## PCT

# WORLD INTELLECTUAL PROPERTY ORGANIZATION



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY-(PCT)

(51) International Patent Classification <sup>6</sup> :	T	(11) International Publication Number:	WO 98/20157
C12Q 1/68	A2	(43) International Publication Date:	14 May 1998 (14.05.98)
(21) International Application Number: PCT/C		BY, CA, CH, CN, CU, CZ, DE,	DK, EE, ES, FI, GB, GE,

(30) Priority Data:
08/743.637 4 November 1996 (04.11.96) US

(71) Applicant (for all designated States except US): INFECTIO DIAGNOSTIC (I.D.I.) INC. [CA/CA]; 42me étage, 2050, boulevard René Lévesque Ouest, Sainte-Foy, Québec GIV 2K8 (CA).

### (72) Inventors; and

- (75) Inventors/Applicants (for US only): BERGERON, Michel, G. [CA/CA]; 2069, nre Brölard, Sillery, Québec GIT IG2 (CA). PICARD, François, J. [CA/CA]; 1245, rue de la Sapinière, Cap-Rouge, Québec GIY 1A1 (CA). OUELLETTE, Marc [CA/CA]; 1035 de Ploermel, Sillery, Québec GIS 381 (CA), ROY, Paul, H. [US/US]; 28, rue Charles Gamier, Lorettwille, Québec CGA 288 (CA).
- (74) Agents: DUBUC, Jean, H. et al.; Goudreau Gage Dubuc & Martineau Walker, The Stock Exchange Tower, Suite 3400, 800 Place Victoria, P.O. Box 242, Montreal, Québec H4Z 1E9 (CA).

1) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, IP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PI, PT, RO, RU, SD, SE, SG, SI, SK, SL, TI, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AA, Z, BY, KG, KZ, MD, RU, TI, TM, Buropean patent (AT, BC, CH, DE, DK, ES, FI, FR, GB, GR, IE, TI, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

### Published

Without international search report and to be republished upon receipt of that report.

(54) Title: SPECIES-SPECIFIC, GENUS-SPECIFIC AND UNIVERSAL DNA PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY COMMON BACTERIAL AND FUNGAL PATHOGENS AND ASSOCIATED ANTIBIOTIC RESISTANCE GENES FROM CLINICAL SPECIMENS FOR DIAGNOSIS IN MICROBIOLOGY LABORATORIES

#### (57) Abstract

DNA—based methods employing amplification primers or probes for detecting, identifying, and quantifying in a test sample DNA for (i) any bacterium, (ii) the species Streptococcus qualacties, Staphylococcus sprephyticus, Enterrococcus faccium, Neisseria meningitidis, Listeria description of the species and Candida albicans, and (ii) any species of the genera Streptococcus, Staphylococcus, Enterococcus, Neisseria and the species of the spe

## FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	Prance	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ.	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	
CA	Canada	IT	Italy	MX	Mexico	UZ	United States of America
CF	Central African Republic	JP	Japan	NE	Niger	VN	Uzbekistan
CG	Congo	KE	Kenya	NL	Netherlands	YU	Vict Nam
CH	Switzerland	KG	Kyrgyzstan	NO	Norway		Yugoslavia
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand	zw	Zimbabwe
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Deumark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

## TITLE OF THE INVENTION

5

10

15

20

25

30

35

SPECIES-SPECIFIC, GENUS-SPECIFIC AND UNIVERSAL DNA PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY COMMON BACTERIAL AND FUNGAL PATHOGENS AND ASSOCIATED ANTIBIOTIC RESISTANCE GENES FROM CLINICAL SPECIMENS FOR DIAGNOSIS IN MICROBIOLOGY LABORATORIES

## BACKGROUND OF THE INVENTION

# Classical methods for the identification and susceptibility testing of bacteria

Bacteria are classically identified by their ability to utilize different substrates as a source of carbon and nitrogen through the use of biochemical tests such as the API20E™ system (bioMérieux). For susceptibility testing, clinical microbiology laboratories use methods including disk diffusion, agar dilution and broth microdilution. Although identifications based on biochemical tests and antibacterial susceptibility tests are cost-effective, at least two days are required to obtain preliminary results due to the necessity of two successive overnight incubations to identify the bacteria from clinical specimens as well as to determine their susceptibility to antimicrobial agents. There are some commercially available automated systems (i.e. the MicroScan system from Dade Diagnostics Corp. and the Vitek system from bioMérieux) which use sophisticated and expensive apparatus for faster microbial identification and susceptibility testing (Stager and Davis, 1992, Clin. Microbiol. Rev. 5:302-327). These systems require shorter incubation periods, thereby allowing most bacterial identifications and susceptibility testing to be performed in less than 6 hours. Nevertheless, these faster systems always require the primary isolation of the bacteria as a pure culture, a process which takes at least 18 hours for a pure culture or 2 days for a mixed culture. The fastest identification system, the autoSCAN-Walk-Away™ system (Dade Diagnostics Corp.) identifies both gram-negative and gram-positive bacterial species from standardized inoculum in as little as 2 hours and gives susceptibility patterns to most antibiotics in 5.5 hours. However, this system has a particularly high percentage (i.e. 3.3 to 40.5%) of non-conclusive identifications with bacterial species other than Enterobacteriaceae (Croizé J., 1995, Lett. Infectiol. 10:109-113; York et al., 1992, J. Clin. Microbiol. 30:2903-2910). For Enterobacteriaceae, the percentage of non-conclusive identifications was 2.7 to 11.4%.

A wide variety of bacteria and fungi are routinely isolated and identified from clinical specimens in microbiology laboratories. Tables 1 and 2 give the incidence for the most commonly isolated bacterial and fungal pathogens from various types of clinical specimens. These pathogens are the most frequently associated with nosocomial and community-acquired human infections and are therefore considered the most clinically important.

10

15

20

25

30

35

# Clinical specim ns test d in clinical microbiology laboratori s

Most clinical specimens received in clinical microbiology laboratories are urine and blood samples. At the microbiology laboratory of the Centre Hospitalier de l'Université Laval (CHUL), urine and blood account for approximately 55% and 30% of the specimens received, respectively (Table 3). The remaining 15% of clinical specimens comprise various biological fluids including sputum, pus, cerebrospinal fluid, synovial fluid, and others (Table 3). Infections of the urinary tract, the respiratory tract and the bloodstream are usually of bacterial etiology and require antimicrobial therapy. In fact, all clinical samples received in the clinical microbiology laboratory are tested routinely for the identification of bacteria and susceptibility testing.

# Conventional pathogen identification from clinical specimens

### Urine specimens

The search for pathogens in urine specimens is so preponderant in the routine microbiology laboratory that a myriad of tests have been developed. However, the gold standard remains the classical semi-quantitative plate culture method in which 1 µL of urine is streaked on plates and incubated for 18-24 hours. Colonies are then counted to determine the total number of colony forming units (CFU) per liter of urine. A bacterial urinary tract infection (UTI) is normally associated with a bacterial count of 10° CFU/L or more in urine. However, infections with less than 10° CFU/L in urine are possible, particularly in patients with a high incidence of diseases or those catheterized (Stark and Maki, 1984, N. Engl. J. Med. 311:560-564). Importantly, approximately 80% of urine specimens tested in clinical microbiology laboratories are considered negative (i.e. bacterial count of less than 10° CFU/L; Table 3). Urine specimens found positive by culture are further characterized using standard biochemical tests to identify the bacterial pathogen and are also tested for susceptibility to antibiotics. The biochemical and susceptibility testing normally require 18-24 hours of incubation.

Accurate and rapid urine screening methods for bacterial pathogens would allow a faster identification of negative specimens and a more efficient treatment and care management of patients. Several rapid identification methods (Uriscreen™, UTIscreen™, Flash Track™ DNA probes and others) have been compared to slower standard biochemical methods, which are based on culture of the bacterial pathogens. Although much faster, these rapid tests showed low sensitivities and poor specificities as well as a high number of false negative and false positive results (Koening *et al.*, 1992, J. Clin. Microbiol. 30:342-345; Pezzlo *et al.*, 1992, J. Clin. Microbiol. 30:640-684).

### **Blood specimens**

The blood specimens received in the microbiology laboratory are always submitted for culture. Blood culture systems may be manual, semi-automated or completely automated. The BACTEC system (from Becton Dickinson) and the

10

15

20

25

30

35

BacTAlert system (from Organon Teknika Corporation) are the two most widely used automated blood culture systems. These systems incubate blood culture bottles under optimal conditions for bacterial growth. Bacterial growth is monitored continuously to detect early positives by using highly sensitive bacterial growth detectors. Once growth is detected, a Gram stain is performed directly from the blood culture and then used to inoculate nutrient agar plates. Subsequently, bacterial identification and susceptibility testing are carried out from isolated bacterial colonies with automated systems as described previously. The bottles are normally reported as negative if no growth is detected after an incubation of 6 to 7 days. Normally, the vast majority of blood cultures are reported negative. For example, the percentage of negative blood cultures at the microbiology laboratory of the CHUL for the period February 1994–January 1995 was 93.1% (Table 3).

## Other clinical samples

Upon receipt by the clinical microbiology laboratory, all body fluids other than blood and urine that are from normally sterile sites (i.e. cerebrospinal, synovial, pleural, pericardial and others) are processed for direct microscopic examination and subsequent culture. Again, most clinical samples are negative for culture (Table 3).

Regarding clinical specimens which are not from sterile sites such as sputum or stool specimens, the laboratory diagnosis by culture is more problematic because of the contamination by the normal flora. The bacterial pathogens potentially associated with the infection are purified from the contaminants and then identified as described previously. Of course, the universal detection of bacteria would not be useful for the diagnosis of bacterial infections at these non sterile sites. On the other hand, DNA-based assays for species or genus detection and identification as well as for the detection of antibiotic resistance genes from these specimens would be very useful and would offer several advantages over classical identification and susceptibility testing methods.

## DNA-based assays with any clinical specimens

There is an obvious need for rapid and accurate diagnostic tests for bacterial detection and identification directly from clinical specimens. DNA-based technologies are rapid and accurate and offer a great potential to improve the diagnosis of infectious diseases (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). The DNA probes and amplification primers which are objects of the present invention are applicable for bacterial or fungal detection and identification directly from any clinical specimens such as blood cultures, blood, urine, sputum, cerebrospinal fluid, pus and other type of specimens (Table 3). The DNA-based tests proposed in this invention are superior in terms of both rapidity and accuracy to standard biochemical methods currently used for routine diagnosis from any clinical specimens in microbiology laboratories. Since

10

15

20

25

30

35

these tests are performed in around only one hour, they provide the clinicians with new diagnostic tools which should contribute to increase the efficiency of therapies with antimicrobial agents. Clinical specimens from organisms other than humans (e.g. other primates, birds, plants, mammals, farm animals, livestock and others) may also be tested with these assays.

# A high percentage of culture negative specimens

Among all the clinical specimens received for routine diagnosis, approximately 80% of urine specimens and even more (around 95%) for other types of clinical specimens are negative for the presence of bacterial pathogens (Table 3). It would also be desirable, in addition to identify bacteria at the species or genus level in a given specimen, to screen out the high proportion of negative clinical specimens with a test detecting the presence of any bacterium (i.e. universal bacterial detection). Such a screening test may be based on the DNA amplification by PCR of a highly conserved genetic target found in all bacteria. Specimens negative for bacteria would not be amplified by this assay. On the other hand, those that are positive for bacteria would give a positive amplification signal with this assay.

# Towards the development of rapid DNA-based diagnostic tests

A rapid diagnostic test should have a significant impact on the management of infections. DNA probe and DNA amplification technologies offer several advantages over conventional methods for the identification of pathogens and antibiotic resistance genes from clinical samples (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.; Ehrlich and Greenberg, 1994, PCR-based Diagnostics in Infectious Disease, Blackwell Scientific Publications, Boston, MA). There is no need for culture of the bacterial pathogens, hence the organisms can be detected directly from clinical samples. thereby reducing the time associated with the isolation and identification of pathogens. Furthermore, DNA-based assays are more accurate for bacterial identification than currently used phenotypic identification systems which are based on biochemical tests. Commercially available DNA-based technologies are currently used in clinical microbiology laboratories, mainly for the detection and identification of fastidious bacterial pathogens such as Mycobacterium tuberculosis, Chlamydia trachomatis, Neisseria gonorrhoeae as well as for the detection of a variety of viruses (Podzorski and Persing, Molecular detection and identification of microorganisms, In: P. Murray et al., 1995, Manual of Clinical Microbiology, ASM press, Washington D.C.). There are also other commercially available DNA-based assays which are used for culture confirmation assays.

Others have developed DNA-based tests for the detection and identification of bacterial pathogens which are objects of the present invention: Staphylococcus spp. (US patent application serial No. US 5 437 978), Neisseria spp. (US patent application

10

15

20

25

30

35

serial No. US 5 162 199 and European patent application serial No. EP 0 337 896 131) and *Listeria monocytogenes* (US patent applications serial Nos US 5 389 513 and US 5 089 386). However, the diagnostic tests described in these patents are based either on rRNA genes or on genetic targets different from those described in the present invention.

Although there are diagnostic kits or methods already used in clinical microbiology laboratories, there is still a need for an advantageous alternative to the conventional culture identification methods in order to improve the accuracy and the speed of the diagnosis of commonly encountered bacterial infections. Besides being much faster, DNA-based diagnostic tests are more accurate than standard biochemical tests presently used for diagnosis because the bacterial genotype (e.g. DNA level) is more stable than the bacterial phenotype (e.g. metabolic level).

Knowledge of the genomic sequences of bacterial and fungal species continuously increases as testified by the number of sequences available from databases. From the sequences readily available from databases, there is no indication therefrom as to their potential for diagnostic purposes. For determining good candidates for diagnostic purposes, one could select sequences for DNA-based assays for (i) the species-specific detection and identification of commonly encountered bacterial or fungal pathogens, (ii) the genus-specific detection and identification of commonly encountered bacterial or fungal pathogens and/or (iv) the specific detection and identification of antibiotic resistance genes. All of the above types of DNA-based assays may be performed directly from any type of clinical specimens or from a microbial culture.

In our co-pending U.S. (N.S. 08/526,840) and PCT (PCT/CA/95/00528) patent applications, we described DNA sequences suitable for (i) the species-specific detection and identification of 12 clinically important bacterial pathogens, (ii) the universal detection of bacteria, and (iii) the detection of 17 antibiotic resistance genes. This co-pending application described proprietary DNA sequences and DNA sequences selected from databases (in both cases, fragments of at least 100 base pairs), as well as oligonucleotide probes and amplification primers derived from thes sequences. All the nucleic acid sequences described in this patent application enter the composition of diagnostic kits and methods capable of a) detecting the presence of bacteria, b) detecting specifically the presence of 12 bacterial species and 17 antibiotic resistance genes. However, these methods and kits need to be improved, since the ideal kit and method should be capable of diagnosing close to 100% of microbial pathogens and antibiotic resistance genes. For example, infections caused by *Enterococcus faecium* have become a clinical problem because of its resistance to many antibiotics. Both the detection of these bacteria and the evaluation of their

resistance profiles are desirable. Besides that, novel DNA sequences (probes and primers) capable of recognizing the same and other microbial pathogens or the same and additional antibiotic resistance genes are also desirable to aim at detecting more target genes and complement our earlier patent application.

5

## STATEMENT OF THE INVENTION

It is an object of the present invention to provide a specific, ubiquitous and sensitive method using probes and/or amplification primers for determining the presence and/or amount of nucleic acids:

10

- from specific microbial species or genera selected from the group consisting of Streptococcus species, Streptococcus agalactiae, Staphylococcus species, Staphylococcus saprophyticus, Enterococcus species, Enterococcus faecium, Neisseria species, Neisseria meningitidis, Listeria monocytogenes, Candida species and Candida albicans

15

- from an antibiotic resistance gene selected from the group consisting of bla<sub>tern</sub>, bla<sub>cos</sub>, bla<sub>sh</sub>, bla<sub>cos</sub>, bla<sub>do</sub>, bla<sub>cos</sub>, bla<sub>do</sub>, bla<sub></sub>

- from any bacterial species

20

in any sample suspected of containing said nucleic acids,

wherein each of said nucleic acids or a variant or part thereof comprises a selected target region hybridizable with said probe or primers;

25

or primers and detecting the presence and/or amount of hybridized probes or amplified products as an indication of the presence and/or amount of said any bacterial species, specific microbial species or genus and antibiotic resistance gene.

In a specific embodiment, a similar method directed to each specific microbial species or genus detection and identification, antibiotic resistance genes detection, and universal bacterial detection, separately, is provided.

30

In a more specific embodiment, the method makes use of DNA fragments (proprietary fragments and fragments obtained from databases), selected for their capacity to sensitively, specifically and ubiquitously detect the targeted bacterial or fungal nucleic acids.

35

In a particularly preferred embodiment, oligonucleotides of at least 12 nucleotides in length have been derived from the longer DNA fragments, and are used in the present method as probes or amplification primers.

The proprietary oligonucleotides (probes and primers) are also another object of the invention.

Diagnostic kits comprising probes or amplification primers for the detection of

98/2015/ - 7 -

a microbial species or genus selected from the group consisting of Streptococcus species, Staphylococcus species, Staphylococcus saprophyticus, Enterococcus species, Enterococcus species, Policy Staphylococcus species, Neisseria meningitidis, Listeria monocytogenes, Candida species and Candida albicans are also objects of the present invention.

Diagnostic kits further comprising probes or amplification primers for the detection of an antibiotic resistance gene selected from the group consisting of bla<sub>tem</sub>, bla<sub>sh</sub>, bla<sub>sh</sub>,

Diagnostic kits further comprising probes or amplification primers for the detection of any bacterial or fungal species, comprising or not comprising those for the detection of the specific microbial species or genus listed above, and further comprising or not comprising probes and primers for the antibiotic resistance genes listed above, are also objects of this invention.

In a preferred embodiment, such a kit allows for the separate or the simultaneous detection and identification of the above-listed microbial species or genus, antibiotic resistance genes and for the detection of any bacterium.

In the above methods and kits, amplification reactions may include a) polymerase chain reaction (PCR), b) ligase chain reaction, c) nucleic acid sequence-based amplification, d) self-sustained sequence replication, e) strand displacement amplification, f) branched DNA signal amplification, g) transcription-mediated amplification. h) cycling probe technology (CPT) i) nested PCR: or i) multiplex PCR:

In a preferred embodiment, a PCR protocol is used as an amplification reaction.

In a particularly preferred embodiment, a PCR protocol is provided, comprising, for each amplification cycle, an annealing step of 30 seconds at 45-55°C and a denaturation step of only one second at 95°C, without any time allowed specifically for the elongation step. This PCR protocol has been standardized to be suitable for PCR reactions with all selected primer pairs, which greatly facilitates the testing because each clinical sample can be tested with universal, species-specific, genus-specific antibiotic resistance gene PCR primers under uniform cycling conditions. Furthermore, various combinations of primer pairs may be used in multiplex PCR assays.

We aim at developing a rapid test or kit to discard rapidly all the samples which are negative for bacterial cells and to subsequently detect and identify the above bacterial and/or fungal species and genera and to determine rapidly the bacterial resistance to antibiotics. Although the sequences from the selected antibiotic resistance genes are available from databases and have been used to develop DNA-based tests for their detection, our approach is unique because it represents a major improvement over current gold standard diagnostic methods based on bacterial

5

10

15

20

25

30

35

10

15

20

25

30

35

cultures. Using an amplification method for the simultaneous bacterial detection and identification and antibiotic resistance genes detection, there is no need for culturing the clinical sample prior to testing. Moreover, a modified PCR protocol has been developed to detect all target DNA sequences in approximately one hour under uniform amplification conditions. This procedure will save lives by optimizing treatment, will diminish antibiotic resistance because less antibiotics will be prescribed, will reduce the use of broad spectrum antibiotics which are expensive, decrease overall health care costs by preventing or shortening hospitalizations, and decrease the time and costs associated with clinical laboratory testing.

In the methods and kits described herein below, the oligonucleotide probes and amplification primers have been derived from larger sequences (i.e. DNA fragments of at least 100 base pairs). All DNA fragments have been obtained either from proprietary fragments or from databases. DNA fragments selected from databases are newly used in a method of detection according to the present invention, since they have been selected for their diagnostic potential.

It is clear to the individual skilled in the art that other oligonucleotide sequences

appropriate for (i) the universal bacterial detection, (ii) the detection and identification of the above microbial species or genus and (iii) the detection of antibiotic resistance genes other than those listed in Annex VI may also be derived from the proprietary fragments or selected database sequences. For example, the oligonucleotide primers or probes may be shorter or longer than the ones we have chosen; they may also be selected anywhere else in the proprietary DNA fragments or in the sequences selected was from databases; they may be also variants of the same oligonucleotide. If the target DNA or a variant thereof hybridizes to a given oligonucleotide, or if the target DNA or a variant thereof can be amplified by a given oligonucleotide PCR primer pair, the converse is also true; a given target DNA may hybridize to a variant oligonucleotide probe or be amplified by a variant oligonucleotide PCR primer. Alternatively, the oligonucleotides may be designed from any DNA fragment sequences for use in amplification methods other than PCR. Consequently, the core of this invention is the identification of universal, species-specific, genus-specific and resistance gene-specific genomic or non-genomic DNA fragments which are used as a source of specific and ubiquitous oligonucleotide probes and/or amplification primers. Although the selection and evaluation of oligonucleotides suitable for diagnostic purposes requires much effort, it is quite possible for the individual skilled in the art to derive, from the selected DNA fragments, oligonucleotides other than the ones listed in Annex VI which are suitable for diagnostic purposes. When a proprietary fragment or a database sequence is selected for its specificity and ubiquity, it increases the probability that subsets thereof will also be specific and ubiquitous.

Since a high percentage of clinical specimens are negative for bacteria (Table

10

15

20

25

30

35

3). DNA fragments having a high potential for the selection of universal oligonucleotide probes or primers were selected from proprietary and database sequences. The amplification primers were selected from a gene highly conserved in bacteria and fungi, and are used to detect the presence of any bacterial pathogen in clinical specimens in order to determine rapidly (approximately one hour) whether it is positive or negative for bacteria. The selected gene, designated tuf, encodes a protein (EF-Tu) involved in the translational process during protein synthesis. The tuf gene seguence alignments used to derive the universal primers include both proprietary and database sequences (Example 1 and Annex I). This strategy allows the rapid screening of the numerous negative clinical specimens (around 80% of the specimens received, see Table 3) submitted for bacteriological testing. Tables 4, 5 and 6 provide a list of the bacterial or fungal species used to test the specificity of PCR primers and DNA probes. Table 7 gives a brief description of each species-specific, genus-specific and universal amplification assays which are objects of the present invention. Tables 8, 9 and 10 provide some relevant information about the proprietary and database sequences selected for diagnostic puposes.

## DETAILED DESCRIPTION OF THE INVENTION

<u>Development of species-specific, genus-specific, universal and antibiotic resistance gene-specific DNA probes and amplification primers for microorganisms</u>

# Selection from databases of sequences suitable for diagnostic purposes

In order to select sequences which are suitable for species-specific or genusspecific detection and identification of bacteria or fungi or, alternatively, for the universal detection of bacteria, the database sequences (GenBank, EMBL and Swiss-Prot) were chosen based on their potential for diagnostic purposes according to sequence information and computer analysis performed with these sequences. Initially, all sequence data available for the targeted microbial species or genus were carefully analyzed. The gene sequences which appeared the most promising for diagnostic purposes based on sequence information and on sequence comparisons with the corresponding gene in other microbial species or genera performed with the Genetics Computer Group (GCG, Wisconsin) programs were selected for testing by PCR. Optimal PCR amplification primers were chosen from the selected database sequences with the help of the Oligo™ 4.0 primer analysis software (National Biosciences Inc., Plymouth, Minn.). The chosen primers were tested in PCR assays for their specificity and ubiquity for the target microbial species or genus. In general, the identification of database sequences from which amplification primers suitable for species-sp cific or genus-specific detection and identification were selected involved the computer analysis and PCR testing of several candidate gene sequences before

10

15

20

25

30

35

obtaining a primer pair which is specific and ubiquitous for the target microbial species or genus. Annex VI provides a list of selected specific and ubiquitous PCR primer pairs. Annexes I to V and Examples 1 to 4 illustrate the strategy used to select genus-specific, species-specific and universal PCR primers from *tuf* sequences or from the *rec*A gene.

# Oligonucleotide primers and probes design and synthesis

The DNA fragments sequenced by us or selected from databases (GenBank and EMBL) were used as sources of oligonucleotides for diagnostic purposes. For this strategy, an array of suitable oligonucleotide primers or probes derived from a variety of genomic DNA fragments (size of more than 100 bp) selected from databases were tested for their specificity and ubiquity in PCR and hybridization assays as described later. It is important to note that the database sequences were selected based on their potential for being species-specific, genus-specific or universal for the detection of bacteria or fungi according to available sequence information and extensive analysis and that, in general, several candidate database sequences had to be tested in order to obtain the desired specificity, ubiquity and sensitivity.

Oligonucleotide probes and amplification primers derived from species-specific fragments selected from database sequences were synthesized using an automated DNA synthesizer (Perkin-Elmer Corp., Applied Biosystems Division). Prior to synthesis, all oligonucleotides (probes for hybridization and primers for DNA amplification) were evaluated for their suitability for hybridization or DNA amplification by polymerase chain reaction (PCR) by computer analysis using standard programs (i.e. the Genetics Computer Group (GCG) programs and the primer analysis software Oligo<sup>™</sup> 4.0). The potential suitability of the PCR primer pairs was also evaluated prior to the synthesis by verifying the absence of unwanted features such as long stretches of one nucleotide and a high proportion of G or C residues at the 3' end (Persing *et al.*, 1993, Diagnostic Molecular Microbiology. Principles and Applications, American Society for Microbiology, Washington, D.C.).

The oligonucleotide primers or probes may be derived from either strand of the duplex DNA. The primers or probes may consist of the bases A, G, C, or T or analogs and they may be degenerated at one or more chosen nucleotide position(s). The primers or probes may be of any suitable length and may be selected anywhere within the DNA sequences from proprietary fragments or from selected database sequences which are suitable for (i) the universal detection of bacteria, (ii) the species-specific detection and identification of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae and Candida albicans (iii) the genus-specific detection of Streptococcus species, Enterococcus species, Staphylococcus species and Neisseria species or (iv) the detection of the 26 above-mentioned clinically important antibiotic resistance genes.

10

15

20

25

30

35

Variants for a given target bacterial gene are naturally occurring and ar attributable to sequence variation within that gene during evolution (Watson et al.. 1987, Molecular Biology of the Gene, 4th ed., The Benjamin/Cummings Publishing Company, Menlo Park, CA; Lewin, 1989, Genes IV, John Wiley & Sons, New York, NY). For example, different strains of the same bacterial species may have a single or more nucleotide variation(s) at the oligonucleotide hybridization site. The person skilled in the art is well aware of the existence of variant bacterial or fungal DNA sequences for a specific gene and that the frequency of sequence variations depends on the selective pressure during evolution on a given gene product. The detection of a variant sequence for a region between two PCR primers may be demonstrated by sequencing the amplification product. In order to show the presence of sequence variants at the primer hybridization site, one has to amplify a larger DNA target with PCR primers outside that hybridization site. Sequencing of this larger fragment will allow the detection of sequence variation at this site. A similar strategy may be applied to show variants at the hybridization site of a probe. Insofar as the divergence of the target sequences or a part thereof does not affect the specificity and ubiquity of the amplification primers or probes, variant bacterial DNA is under the scope of this invention. Variants of the selected primers or probes may also be used to amplify or hybridize to a variant DNA.

# Sequencing of tuf sequences from a variety of bacterial and fungal species

The nucleotide sequence of a portion of tuf genes was determined for a variety of bacterial and fungal species. The amplification primers SEQ ID NOs: 107 and 108, which amplify a tuf gene portion of approximately 890 bp, were used for the sequencing of bacterial tuf sequences. The amplification primers SEQ ID NOs: 109 and 172, which amplify a tuf gene portion of approximately 830 bp, were used for the sequencing of fungal tuf sequences. Both primer pairs can amplify tufA and tufB genes. This is not surprising because these two genes are nearly identical. For example, the entire tufA and tufB genes from E. coli differ at only 13 nucleotide positions (Neidhardt et al., 1996, Escherichia coli and Salmonella: Cellular and Molecular Biology, 2nd ed., American Society for Microbiology Press, Washington, D.C.). These amplification primers are degenerated at several nucleotide positions and contain inosines in order to allow the amplification of a wide range of tuf sequences. The strategy used to select these amplification primers is similar to that illustrated in Annex I for the selection of universal primers. The amplification primers SEQ ID NOs: 107 and 108 could be used to amplify the tuf genes from any bacterial species. The amplification primers SEQ ID NOs: 109 and 172 could be used to amplify the tuf gen s from any fungal species.

The tuf genes were amplified directly from bacterial or yeast cultures using the following amplification protocol: One  $\mu L$  of cell suspension was transferred directly to

19  $\mu$ L of a PCR reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl<sub>2</sub>, 1  $\mu$ M of each of the 2 primers, 200  $\mu$ M of each of the four dNTPs, 0.5 unit of Taq DNA polymerase (Promega Corp., Madison, WI). PCR reactions were subjected to cycling using a MJ Research PTC-200 thermal cycler (MJ Research Inc., Watertown, Mass.) as follows: 3 min at 96°C followed by 30-35 cycles of 1 min at 95°C for the denaturation step, 1 min at 30-50°C for the annealing step and 1 min at 72°C for the extension step. Subsequently, twenty microliters of the PCRamplified mixture were resolved by electrophoresis in a 1.5% agarose gel. The gel was then visualized by staining with methylene blue (Flores et al., 1992, Biotechniques. 13:203-205). The size of the amplification products was estimated by comparison with a 100-bp molecular weight ladder. The band corresponding to the specific amplification product (i.e. approximately 890 or 830 bp for bacterial or fungal tuf sequences, respectively) was excised from the agarose gel and purified using the QIAquick™ gel extraction kit (QIAGEN Inc., Chatsworth, CA). The gel-purified DNA fragment was then used directly in the sequencing protocol. Both strands of the tuf genes amplification product were sequenced by the dideoxynucleotide chain termination sequencing method by using an Applied Biosystems automated DNA sequencer (model 373A) with their PRISM™ Sequenase® Terminator Double-stranded DNA Sequencing Kit (Perkin-Elmer Corp., Applied Biosystems Division, Foster City, CA). The sequencing reactions were all performed by using the amplification primers (SEQ ID NOs: 107 to 109 and 172) and 100 ng per reaction of the gel-purified amplicon. In order to ensure that the determined sequence did not contain errors attributable to the sequencing of PCR artefacts, we have sequenced two preparations of the gel-purified tuf amplification product originating from two independent PCR amplifications. For all target microbial species, the sequences determined for both amplicon preparations were identical. Furthermore, the sequences of both strands were 100% complementary thereby confirming the high accuracy of the determined sequence. The tuf sequences determined using the above strategy are all in the Sequence Listing (i.e. SEQ ID NOs:118 to 146). Table 13 gives the originating microbial species and the source for each tuf sequence in the Sequence Listing.

The alignment of the tuf sequences determined by us or selected from databases reveals clearly that the length of the sequenced portion of the tuf genes is variable. There may be insertions or deletions of several amino acids. This explains why the size of the sequenced tuf amplification product was variable for both bacterial and fungal species. Among the tuf sequences determined by our group, we found insertions and deletions adding up to 5 amino acids or 15 nucleotides. Consequently, the nucleotide positions indicated on top of each of Annexes I to V do not correspond for tuf sequences having insertions or deletions.

It should also be noted that the various tuf sequences determined by us

5

10

15

20

25

30

35

10

15

20

25

30

35

occasionally contain degenerescences. These degenerated nucleotides correspond to sequence variations between *tufA* and *tufB* genes because the amplification primers amplify both *tuf* genes. These nucleotide variations were not attributable to nucleotide misincorporations by the *taq* DNA polymerase because the sequence of both strands were identical and also because the sequences determined with both preparations of the gel-purified *tuf* amplicons were identical.

## The selection of amplification primers from tuf sequences

The *tuf* sequences determined by us or selected from databases were used to select PCR primers for (i) the universal detection of bacteria, (ii) the genus-specific detection and identification of *Enterococcus* spp. and *Staphylococcus* spp. and (iii) the species-specific detection and identification of *Candida albicans*. The strategy used to select these PCR primers was based on the analysis of multiple sequence alignments of various *tuf* sequences. For more details about the selection of PCR primers from *tuf* sequences, please refer to Examples 1 to 3 and Annexes I to IV.

## The selection of amplification primers from recA

The comparison of the nucleotide sequence for the *recA* gene from various bacterial species including 5 species of streptococci allowed the selection of *Streptococcus*-specific PCR primers. For more details about the selection of PCR primers from *recA*, please refer to Example 4 and Annex V.

# <u>DNA fragment isolation from Staphylococcus saprophyticus by arbitrarily primed PCR</u>

DNA sequences of unknown coding potential for the species-specific detection and identification of Staphylococcus saprophyticus were obtained by the method of arbitrarily primed PCR (AP-PCR).

AP-PCR is a method which can be used to generate specific DNA probes for microorganisms (Fani et al., 1993, Mol. Ecol. 2:243-250). A description of the AP-PCR protocol used to isolate a species-specific genomic DNA fragment from Staphylococcus saprophyticus follows. Twenty different oligonucleotide primers of 10 nucleotides in length (all included in the AP-PCR kit OPAD (Operon Technologies, Inc., Alameda, CA)) were tested systematically with DNAs from 3 bacterial strains of Staphylococcus saprophyticus (all obtained from the American Type Culture Collection (ATCC): numbers 15305, 35552 and 43867) as well as with DNA from four other staphylococcus species (Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 14990, Staphylococcus haemolyticus ATCC 29970 and Staphylococcus hominis ATCC 35982). For all bacterial species, amplification was performed from a bacterial suspension adjusted to a standard 0.5 McFarland which corresponds to approximately 1.5 x 10<sup>6</sup> bacteria/mL. One μL of the standardized bacterial suspension was transferred directly to 19 μL of a PCR reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl<sub>2</sub>.

10

15

20

25

30

35

1.2  $\mu$ M of only one of the 20 different AP-PCR primers OPAD, 200  $\mu$ M of each of the four dNTPs and 0.5 unit of Taq DNA polymerase (Promega Corp., Madison, WI). PCR reactions were subjected to cycling using a MJ Research PTC-200 thermal cycler (MJ Research Inc.) as follows: 3 min at 96°C followed by 35 cycles of 1 min at 95°C for the denaturation step, 1 min at 32°C for the annealing step and 1 min at 72°C for the extension step. A final extension step of 7 min at 72°C was made after the 35 cycles to ensure complete extension of PCR products. Subsequently, twenty microliters of the PCR amplified mixture were resolved by electrophoresis in a 2% agarose gel containing 0.25  $\mu$ g/mL of ethicium bromide. The size of the amplification products was estimated by comparison with a 50-bp molecular weight ladder.

Amplification patterns specific for Staphylococcus saprophyticus were observed with the AP-PCR primer OPAD-9 (SEQ ID NO: 25). Amplification with this primer consistently showed a band corresponding to a DNA fragment of approximately 450 bp for all Staphylococcus saprophyticus strains tested but not for any of the four other staphylococcul species tested. This species-specific pattern was confirmed by testing 10 more clinical isolates of S. saprophyticus selected from the culture collection of the microbiology laboratory of the CHUL as well as strains selected from the gram-positive bacterial species listed in Table 5.

The band corresponding to the approximately 450 bp amplicon which was specific and ubiquitous for *S. saprophyticus* based on AP-PCR was excised from the agarose gel and purified using the QIAquick™ gel extraction kit (QIAGEN Inc.). The gel-purified DNA fragment was cloned into the T/A cloning site of the pCR 2.1™ plasmid vector-(Invitrogen-Inc.)-using-T4 DNA-ligase (New-England-BioLabs).—Recombinant plasmids were transformed into *E. coli* DH5α competent cells using standard procedures. Plasmid DNA isolation was done by the method of Birnboim and Doly (Nucleic Acids Res. 7:1513-1523) for small-scale preparations. All plasmid DNA preparations were digested with the *Eco*RI restriction endonuclease to ensure the presence of the approximately 450 bp AP-PCR insert into the recombinant plasmids. Subsequently, a large-scale and highly purified plasmid DNA preparation was performed from two selected clones shown to carry the AP-PCR insert by using the QIAGEN plasmid purification kit. These plasmid preparations were used for automated DNA sequencing.

Both strands of the AP-PCR insert from the two selected clones were sequenced by the dideoxynucleotide chain termination sequencing method with SP6 and T7 sequencing primers, by using an Applied Biosystems automated DNA sequencer as described previously. The analysis of the obtained sequences revealed that the DNA sequences for both strands from each clone were 100% complementary. Furthermore, it showed that the entire sequence determined for each clone were both identical. These sequencing data confirm the 100% accuracy for the determined 438

10

15

20

25

30

35

bp sequence (SEQ ID NO: 29). Optimal amplification primers have been selected from the sequenced AP-PCR Staphylococcus saprophyticus DNA fragment with the help of the primer analysis software Oligo™ 4.0. The selected primer sequences have been tested in PCR assays to verify their specificity and ubiquity (Table 7). These PCR primers were specific since there was no amplification with DNA from bacterial species other than S. saprophyticus selected from Tables 4 and 5. Furthermore, this assay was ubiquitous since 245 of 260 strains of S. saprophyticus were efficiently amplified with this PCR assay. When used in combination with another S. saprophyticus-specific PCR assay, which is an object of our co-pending U.S. (N.S. 08/526,840) and PCT (PCT/CA/95/00528) patent applications, the ubiquity reaches 100% for these 260 strains.

## **DNA** amplification

For DNA amplification by the widely used PCR (polymerase chain reaction) method, primer pairs were derived from proprietary DNA fragments or from database sequences. Prior to synthesis, the potential primer pairs were analyzed by using the Oligo TM 4.0 software to verify that they are good candidates for PCR amplification.

During DNA amplification by PCR, two oligonucleotide primers binding respectively to each strand of the heat-denatured target DNA from the bacterial genome are used to amplify exponentially *in vitro* the target DNA by successive thermal cycles allowing denaturation of the DNA, annealing of the primers and synthesis of new targets at each cycle (Persing *et al.*, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology.

Briefly, the PCR protocols were as follow: Treated clinical specimens or standardized bacterial or fungal suspensions (see below) were amplified in a 20  $\mu$ L PCR reaction mixture containing 50 mM KCI, 10 mM Tris-HCI (pH 9.0), 2.5 mM MgCl<sub>2</sub>, 0.4  $\mu$ M of each primer, 200  $\mu$ M of each of the four dNTPs and 0.5 unit of Taq DNA polymerase (Promega) combined with the TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto, CA). The TaqStart™ antibody, which is a neutralizing monoclonal antibody to Tag DNA polymerase, was added to all PCR reactions to enhance the specificity and the sensitivity of the amplifications (Kellogg et al., 1994, Biotechniques 16:1134-1137). The treatment of the clinical specimens varies with the type of specimen tested, since the composition and the sensitivity level required are different for each specimen type. It consists in a rapid protocol to lyse the bacterial cells and eliminate the PCR inhibitory effects (see example 11 for urine specimen preparation). For amplification from bacterial or fungal cultures, the samples were added directly to the PCR amplification mixture without any pre-treatment step (see example 10). Primer sequences derived from highly conserved regions of the bacterial 16S ribosomal RNA gene were used to provide an internal control for all PCR reactions. Alternatively, the - 16 -

internal control was derived from sequences not found in microorganisms or in the human genome. The internal control was integrated into all amplification reactions to verify the efficiency of the PCR assays and to ensure that significant PCR inhibition was absent. The internal control derived from rRNA was also useful to monitor the efficiency of bacterial lysis protocols.

PCR reactions were then subjected to thermal cycling (3 min at 95°C followed by 30 cycles of 1 second at 95°C for the denaturation step and 30 second at 55°C for the annealing-extension step) using a PTC-200 thermal cycler (MJ Research Inc.) and subsequently analyzed by standard ethidium bromide-stained agarose gel electrophoresis. The number of cycles performed for the PCR assays varies according to the sensitivity level required. For example, the sensitivity level required for microbial detection directly from clinical specimens is higher for blood specimens than for urine specimens because the concentration of microorganisms associated with a septicemia can be much lower than that associated with a urinary tract infection. Consequently, more sensitive PCR assays having more thermal cycles are required for direct detection from blood specimens. Similarly, PCR assays performed directly from bacterial or fungal cultures may be less sensitive than PCR assays performed directly from clinical specimens because the number of target organisms is normally much lower in clinical specimens than in microbial cultures.

It is clear that other methods for the detection of specific amplification products. which may be faster and more practical for routine diagnosis, may be used. Such methods may be based on the detection of fluorescence after amplification (e.g. TagMan™ system from Perkin-Elmer or Amplisensor™ from Biotronics). Methods based on the detection of fluorescence are particularly promising for utilization in routine diagnosis as they are very rapid, quantitative and can be automated (Example 14).

Microbial pathogens detection and identification may also be performed by solid support or liquid hybridization using species-specific internal DNA probes hybridizing to an amplification product. Such probes may be generated from any species-specific or genus-specific DNA amplification products which are objects of the present invention. Alternatively, the internal probes for species or genus detection and identification may be derived from the amplicons produced by the universal amplification assay. The oligonucleotide probes may be labeled with biotin or with digoxigenin or with any other reporter molecules.

To assure PCR efficiency, glycerol, dimethyl sulfoxide (DMSO) or other related solvents can be used to increase the sensitivity of the PCR and to overcome problems associated with the amplification of a target DNA having a high GC content or forming strong secondary structures (Dieffenbach and Dveksler, 1995, PCR Primer : A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, New York), The

5

10

15

20

25

30

35

10

15

20

25

30

35

concentration ranges for glycerol and DMSO are 5-15% (v/v) and 3-10% (v/v), respectively. For the PCR reaction mixture, the concentration ranges for the amplification primers and MgCl<sub>2</sub> are 0.1-1.5  $\mu$ M and 1.5-3.5 mM, respectively. Modifications of the standard PCR protocol using external and nested primers (i.e. nested PCR) or using more than one primer pair (i.e. multiplex PCR) may also be used (Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). For more details about the PCR protocols and amplicon detection methods, see Examples 9 to 14.

The person skilled in the art of DNA amplification knows the existence of other rapid amplification procedures such as ligase chain reaction (LCR), transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), branched DNA (bDNA) and cycling probe technology (CPT) (Lee et al., 1997, Nucleic Acid Amplification Technologies: Application to Disease Diagnosis, Eaton Publishing, Boston, MA; Persing et al., 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C.). The scope of this invention is not limited to the use of amplification by PCR, but rather includes the use of any rapid nucleic acid amplification method or any other procedure which may be used to increase rapidity and sensitivity of the tests. Any oligonucleotide suitable for the amplification of nucleic acids by approaches other than PCR and derived from the species-specific, genus-specific and universal DNA fragments as well as from selected antibiotic resistance gene sequences included in this document are also under the

## Hybridization assays with oligonucleotide probes

In hybridization experiments, single-stranded oligonucleotides (size less than 100 nucleotides) have some advantages over DNA fragment probes for the detection of bacteria, such as ease of synthesis in large quantities, consistency in results from batch to batch and chemical stability. Briefly, for the hybridizations, oligonucleotides were 5' end-labeled with the radionucleotide γ-<sup>32</sup>P(dATP) using T4 polynucleotide kinase (Pharmacia) (Sambrook *et al.*, 1988, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). The unincorporated radionucleotide was removed by passing the labeled oligonucleotide through a Sephadex G-50™ column. Alternatively, oligonucleotides were labeled with biotin, either enzymatically at their 3' ends or incorporated directly during synthesis at their 5' ends, or with digoxigenin. It will be appreciated by the person skilled in the art that labeling means other than the three above labels may be used.

Each oligonucleotide probe was then tested for its specificity by hybridization to DNAs from a variety of bacterial and fungal species selected from Tables 4, 5 and 6. All of the bacterial or fungal species tested were likely to be pathogens associated

10

15

20

25

30

35

with common infections or potential contaminants which can be isolated from clinical specimens. Each target DNA was released from bacterial cells using standard chemical treatments to lyse the cells (Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor. NY). Subsequently, the DNA was denatured by conventional methods and then irreversibly fixed onto a solid support (e.g. nylon or nitrocellulose membranes) or free in solution. The fixed single-stranded target DNAs were then hybridized with the oligonucleotide probe cells (Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), Prehvbridization conditions were in 1 M NaCl + 10% dextran sulfate + 1% SDS + 100 μα/mL salmon sperm DNA at 65°C for 15 min. Hybridization was performed in fresh pre-hybridization solution containing the labeled probe at 65°C overnight. Posthybridization washing conditions were as follows: twice in 3X SSC containing 1% SDS, twice in 2X SSC containing 1% SDS and twice in 1X SSC containing 1% SDS (all of these washes were at 65°C for 15 min), and a final wash in 0.1X SSC containing 1% SDS at 25°C for 15 min. Autoradiography of washed filters allowed the detection of selectively hybridized probes. Hybridization of the probe to a specific target DNA indicated a high degree of similarity between the nucleotide sequence of these two DNAs because of the high stringency of the washes.

An oligonucleotide probe was considered specific only when it hybridized solely to DNA from the species or genus from which it was isolated. Oligonucleotide probes found to be specific were subsequently tested for their ubiquity (i.e. ubiquitous probes recognized-most or all-isolates of the target species or genus) by hybridization to microbial DNAs from clinical isolates of the species or genus of interest including ATCC strains. The DNAs from strains of the target species or genus were denatured, fixed onto nylon membranes and hybridized as described above. Probes were considered ubiquitous when they hybridized specifically with the DNA from at least 80% of the isolates of the target species or genus.

Specificity and ubiquity tests for oligonucleotide primers and probes

The specificity of oligonucleotide primers and probes, derived either from the DNA fragments sequenced by us or selected from databases, was tested by amplification of DNA or by hybridization with bacterial or fungal species selected from those listed in Tables 4, 5 and 6, as described in the two previous sections. Oligonucleotides found to be specific were subsequently tested for their ubiquity by amplification (for primers) or by hybridization (for probes) with bacterial DNAs from isolates of the target species or genus. Results for specificity and ubiquity tests with the oligonucleotide primers are summarized in Table 7. The specificity and ubiquity of the PCR assays using the selected amplification primer pairs were tested directly from cultures (see Examples 9 and 10) of bacterial or fungal species.

10

15

20

25

30

35

The various species-specific and genus-specific PCR assays which are objects of the present invention are all specific. For the PCR assays specific to bacterial species or genus, this means that DNA isolated from a wide variety of bacterial species, other than that from the target species or genus and selected from Tables 4 and 5, could not be amplified. For the PCR assay specific to Candida albicans, it means there was no amplification with genomic DNA from the fungal species listed in Table 6 as well as with a variety of bacterial species selected from Tables 4 and 5.

The various species-specific and genus-specific PCR assays which are objects of the present invention are also all ubiquitous (Table 7). (i) The species-specific PCR assays for E. faecium, L. monocytogenes, S. saprophyticus, S. agalactiae and C. albicans amplified genomic DNA from all or most strains of the target species tested, which were obtained from various sources and which are representative of the diversity within each target species (Table 7). The species identification of all of these strains was based on classical biochemical methods which are routinely used in clinical microbiology laboratories. (ii) The genus-specific PCR assays specific for Enterococcus spp., Staphylococcus spp., Streptococcus spp. and Neisseria spp. amplified genomic DNA from all or most strains of the target genus tested, which represent all clinically important bacterial species for each target genus. These strains were obtained from various sources and are representative of the diversity within each target genus. Again, the species identification of all of these strains was based on classical biochemical methods which are routinely used in clinical microbiology laboratories. More specifically, the four genus-specific PCR assays amplified the following species: (1) The Enterococcus-specific assay amplified efficiently-DNA from all of the 11 enterococcal species tested including E. avium, E. casseliflavus, E. dispar, E. durans, E. faecalis, E. faecium, E. flavescens, E. gallinarum, E. hirae, E. mundtii and E. raffinosus. (2) The Neisseria-specific assay amplified efficiently DNA from all of the 12 neisserial species tested including N. canis, N. cinerea, N. elongata, N. flavescens, N. gonorrhoeae, N. lactamica, N. meningitidis, N. mucosa, N. polysaccharea, N. sicca, N. subflava and N. weaveri. (3) The Staphylococcus-specific assay amplified efficiently DNA from 13 of the 14 staphylococcal species tested S. aureus, S. auricularis, S. capitis, S. cohnii, S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis, S. saprophyticus, S. schleiferi, S. simulans, S. warneri and S. xylosus. The staphylococcal species which could not be amplified is S. sciuri. (4) Finally, the Streptococcus-specific assay amplified efficiently DNA from all of the 22 streptococcal species tested including S. agalactiae, S. anginosus, S. boyis, S. constellatus, S. crista, S. dysgalactiae, S. equi, S. gordonii, S. intermedius, S. mitis, S. mutans, S. oralis, S. parasanguis, S. pneumoniae, S. pyogenes, S. salivarius, S. sanguis, S. sabrinus, S. suis, S. uberis, S. vestibularis and S. viridans. On the other hand, the Streptococcus-specific assay did not amplify 3 out of 9 strains

10

15

20

25

30

35

of *S. mutans* and 1 out of 23 strains of *S. salivarius*, thereby showing a slight lack of ubiquity for these two streptococcal species.

All specific and ubiquitous amplification primers for each target microbial species or genus or antibiotic resistance gene investigated are listed in Annex VI. Divergence in the sequenced DNA fragments can occur, insofar as the divergence of these sequences or a part thereof does not affect the specificity of the probes or amplification primers. Variant bacterial DNA is under the scope of this invention.

The PCR amplification primers listed in Annex VI were all tested for their specificity and ubiquity using reference strains as well as clinical isolates from various geographical locations. The 351 reference strains used to test the amplification and hybridization assays (Tables 4, 5 and 6) were obtained from (i) the American Type Culture Collection (ATCC): 85%, (ii) the Laboratoire de santé publique du Québec (LSPQ): 10%, (iii) the Centers for Disease Control and Prevention (CDC): 3%, (iv) the National Culture Type Collection (NCTC): 1% and (v) several other reference laboratories throughout the world: 1%. These reference strains are representative of (i) 90 gram-negative bacterial species (169 strains; Table 4), (ii) 97 gram-positive bacterial species (154 strains; Table 5) and (iii) 12 fungal species (28 strains; Table 6). Antiblotic resistance genes

Antimicrobial resistance complicates treatment and often leads to therapeutic failures. Furthermore, overuse of antibiotics inevitably leads to the emergence of bacterial resistance. Our goal is to provide clinicians, in approximately one hour, the needed information to prescribe optimal treatments. Besides the rapid identification of negative clinical specimens with DNA-based tests for universal bacterial detection andthe identification of the presence of a specific pathogen in the positive specimens with species- and/or genus-specific DNA-based tests, clinicians also need timely information about the ability of the bacterial pathogen to resist antibiotic treatments. We feel that the most efficient strategy to evaluate rapidly bacterial resistance to antimicrobials is to detect directly from the clinical specimens the most common and clinically important antibiotic resistance genes (i.e. DNA-based tests for the detection of antibiotic resistance genes). Since the sequence from the most important and common bacterial antibiotic resistance genes are available from databases, our strategy was to use the sequence from a portion or from the entire resistance gene to design specific oligonucleotide primers or probes which will be used as a basis for the development of rapid DNA-based tests. The sequence from each of the bacterial antibiotic resistance genes selected on the basis of their clinical relevance (i.e. high incidence and importance) is given in the Sequence Listing. Tables 9 and 10 summarize some characteristics of the selected antibiotic resistance genes. Our approach is unique because the antibiotic resistance genes detection and the bacterial detection and identification are performed simultaneously in multiplex assays under

10

15

20

25

30

35

uniform PCR amplification conditions (Example 13).

Annex VI provides a list of all amplification primers selected from 26 clinically important antibiotic resistance genes which were tested in PCR assays. The various PCR assays for antibiotic resistance genes detection and identification were validated by testing several resistant bacterial isolates known to carry the targeted gene and obtained from various countries. The testing of a large number of strains which do not carry the targeted resistance gene was also performed to ensure that all assays were specific. So far, all PCR assays for antibiotic resistance genes are highly specific and have detected all control resistant bacterial strains known to carry the targeted gene. The results of some clinical studies to validate the array of PCR assays for the detection and identification of antibiotic resistance genes and correlate these DNAbased assays with standard antimicrobials susceptibility testing methods are presented in Tables 11 and 12.

# Universal bacterial detection

In the routine microbiology laboratory, a high percentage of clinical specimens sent for bacterial identification are negative by culture (Table 4). Testing clinical samples with universal amplification primers or universal probes to detect the presence of bacteria prior to specific identification and screen out the numerous negative specimens is thus useful as it saves costs and may rapidly orient the clinical management of the patients. Several amplification primers and probes were therefore synthesized from highly conserved portions of bacterial sequences from the tuf genes (Table 8). The universal primer selection was based on a multiple sequence alignment constructed with sequences determined by us or selected from available database. sequences as described in Example 1 and Annex I.

For the identification of database sequences suitable for the universal detection of bacteria, we took advantage of the fact that the complete genome sequences for two distant microorganisms (i.e. Mycoplasma genitalium and Haemophilus influenzae) are available. A comparison of the amino acid sequence for all proteins encoded by the genome of these two distant microorganisms led to the identification of highly homologous proteins. An analysis of these homologous proteins allowed to select some promising candidates for the development of universal DNA-based assays for the detection of bacteria. Since the complete nucleotide sequence of several other microbial genomes are presently available in databases, a person skilled in the art could arrive to the same conclusions by comparing genomes sequences other than those of Mycoplasma genitalium and Haemophilus influenzae. The selected tuf gene encodes a protein (EF-Tu) involved in the translation process during protein synthesis. Subsequently, an extensive nucleotide sequence analysis was performed with the tuf gene sequences available in databases as well as with novel tuf sequences which w have determined as described previously. All computer analysis of amino acid and

10

15

20

25

30

35

nucleotide sequences were performed by using the GCG programs. Subsequently, optimal PCR primers for the universal amplification of bacteria were selected with the help of the  $Oligo^{TM}$  program. The selected primers are degenerated at several nucleotide positions and contain several inosines in order to allow the amplification of all clinically relevant bacterial species (Annex I). Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Degenerated oligonucleotides consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches. The inclusion of inosine and/or of degenerescences in the amplification primers allow mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, NY).

The amplification conditions with the universal primers were identical to those used for the species- and genus-specific amplification assays except that the annealing temperature was 50°C instead of 55°C. This universal PCR assay was specific and nearly ubiquitous for the detection of bacteria. The specificity for bacteria was verified by amplifying genomic DNA isolated from the 12 fungal species listed in Table 6 as well as genomic DNA from Leishmania donovani, Saccharomyces cerevisiae and human lymphocytes. None of the above eukaryotic DNA preparations could be amplified by the universal assay, thereby suggesting that this test is specific for bacteria. The ubiquity of the universal assay was verified by amplifying genomic DNAs from 116 reference strains which represent 95 of the most clinically relevant bacterial species. These species have been selected from the bacterial species listed in Tables 4 and 5. We found that 104 of these 116 strains could be amplified. The bacterial species which could not be amplified belong to the following genera: Corynebacterium (11 species) and Stenotrophomonas (1 species). Sequencing of the tuf genes from these bacterial species has been recently performed. This sequencing data has been used to select new universal primers which may be more ubiquitous. These primers are in the process of being tested. We also observed that for several species the annealing temperature had to be reduced to 45°C in order to get an efficient amplification. These bacterial species include Gemella morbilbrum, Listeria spp. (3 species) and Gardnerella vaginalis. It is important to note that the 95 bacterial species selected from Tables 4 and 5 to test the ubiquity of the universal assay include all of the most clinically relevant bacterial species associated with a variety of human infections acquired in the community or in hospitals (nosocomial infections). The most clinically important bacterial and fungal pathogens are listed in Tables 1 and 2.

10

15

20

25

30

35

## EXAMPLES AND ANNEXES

The following examples and annexes are intended to be illustrative of the various methods and compounds of the invention, rather than limiting the scope thereof.

The various annexes show the strategies used for the selection of amplification primers from tuf sequences or from the recA gene: (i) Annex I illustrates the strategy used for the selection of the universal amplification primers from tuf sequences. (ii) Annex II shows the strategy used for the selection of the amplification primers specific for the genus Enterococcus from tuf sequences. (iii) Annex III illustrates the strategy used for the selection of the amplification primers specific for the genus Staphylococcus from tuf sequences. (iv) Annex IV shows the strategy used for the selection of the amplification primers specific for the species Candida albicans from tuf sequences. (v) Annex V illustrates the strategy used for the selection of the amplification primers specific for the genus Streptococcus from recA sequences. (vi) Annex VI gives a list of all selected primer pairs. As shown in these annexes, the selected amplification primers may contain inosines and/or degenerescences. Inosine is a nucleotide analog able to specifically bind to any of the four nucleotides A, C, G or T. Alternatively, degenerated oligonucleotides which consist of an oligonucleotide mix having two or more of the four nucleotides A, C, G or T at the site of mismatches were used. The inclusion of inosine and/or of degenerescences in the amplification primers allow mismatch tolerance thereby permitting the amplification of a wider array of target nucleotide sequences (Dieffenbach and Dveksler, 1995 PCR Primer: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, New York). **EXAMPLES** 

## FXAMPLE 1:

Selection of universal PCR primers from tut sequences. As shown in Annex I, the comparison of tut sequences from a variety of bacterial and eukaryotic species allowed the selection of PCR primers which are universal for the detection of bacteria. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various tut sequences. This multiple sequence alignment includes tut sequences from 38 bacterial species and 3 eukaryotic species either determined by us or selected from databases (Table 13). A careful analysis of this multiple sequence alignment allowed the selection of primer sequences which are conserved within eubacteria but which discriminate sequences from eukaryotes, thereby permitting the universal detection of bacteria. As shown in Annex I, the selected primers contain several inosines and degenerescences. This was necessary because there is a relatively high polymorphism among bacterial tut sequences despite the fact that this gene is highly conserved. In fact, among the tut sequences that we determined, we found many nucleotide variations as well as some deletions and/or

insertions of amino acids. The selected universal primers were specific and ubiquitous for bacteria (Table 7). Of the 95 most clinically important bacterial species tested, 12 were not amplified. These species belong to the genera *Corynebacterium* (11 species) and *Stenotrophomonas* (1 species). The universal primers did not amplify DNA of non-bacterial origin, including human and other types of eukaryotic DNA.

### EXAMPLE 2:

5

10

15

20

25

30

35

Selection of genus-specific PCR primers from tuf sequences. As shown in Annexes 2 and 3, the comparison of tuf sequences from a variety of bacterial species allowed the selection of PCR primers specific for Enterococcus spp. or for Staphylococcus spp. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various tuf sequences. These multiple sequence alignments include the tuf sequences of four representative bacterial species selected from each target genus as well as tuf sequences from species of other closely related bacterial genera. A careful analysis of those alignments allowed the selection of oligonucleotide sequences which are conserved within the target genus but which discriminate sequences from other closely related genera, thereby permitting the genus-specific and ubiquitous detection and identification of the target bacterial genus.

For the selection of primers specific for Enterococcus spp. (Annex II), we have sequenced a portion of approximately 890 bp of the tuf genes for Enterococcus avium, E. faecalis, E. faecium and E. gallinarum. All other tuf sequences used in the alignment were either sequenced by us or selected from databases. The analysis of this sequence alignment led to the selection of a primer pair specific and ubiquitous for Enterococcus spp::(Table:7):All of the 11 enterococcus species tested were efficiently amplified and there was no amplification with genomic DNA from bacterial species of other genera.

For the selection of primers specific for Staphylococcus spp. (Annex III), we have also sequenced a portion of approximately 890 bp of the tuf genes for Staphylococcus aureus, S. epidermidis, S. saprophyticus and S. simulans. All other tuf sequences used in the alignment were either sequenced by us or selected from databases. The analysis of this sequence alignment led to the selection of two primer pairs specific and ubiquitous for Staphylococcus spp. (Table 7). Annex III shows the strategy used to select one of these two PCR primer pairs. The same strategy was used to select the other primer pair. Of the 14 staphylococcul species tested, one (S. sciuri) could not be amplified by the Staphylococcus-specific PCR assays using either one of these two primer pairs. For PCR assays using either one of these two primer pairs, there was no amplification with DNA from species of other bacterial genera.

10

15

20

25

30

35

### **EXAMPLE 3:**

Selection from tuf sequences of PCR primers specific for Candida albicans. As shown in Annex IV, the comparison of tuf sequences from a variety of bacterial and eukaryotic species allowed the selection of PCR primers specific for Candida albicans. The strategy used to design the PCR primers was based on the analysis of a multiple sequence alignment of various tuf sequences. This multiple sequence alignment includes tuf sequences of five representative fungal species selected from the genus Candida which were determined by our group (i.e. C. albicans, C. glabrata, C. krusei, C. parapsilosis and C. tropicalis) as well as tuf sequences from other closely related fungal species. tuf sequences from various bacterial species were also included. A careful analysis of this sequence alignment allowed the selection of primers from the C. albicans tuf sequence; these primers discriminate sequences from other closely related Candida species and other fungal species, thereby permitting the species-specific and ubiquitous detection and identification of C. albicans (Table 7). All of 88 Candida albicans strains tested were efficiently amplified and there was no amplification with genomic DNA from other fungal or bacterial species.

### **EXAMPLE 4:**

Selection of PCR primers specific for Streptococcus from recA. As shown in Annex V, the comparison of the various bacterial recA gene sequences available from databases (GenBank and EMBL) was used as a basis for the selection of PCR primers which are specific and ubiquitous for the bacterial genus Streptococcus. Since sequences of the recA gene are available for many bacterial species including five species of streptococcus but distinct from the recA sequences well conserved within the genus Streptococcus but distinct from the recA sequences for other bacterial genera. When there were mismatches between the recA gene sequences from the five Streptococcus species, an inosine residue was incorporated into the primer (Annex V). The selected primers, each containing one inosine and no degenerescence, were specific and ubiquitous for Streptococcus species (Table 7). This PCR assay amplified all of the 22 streptococcul species tested. However, the Streptococcus-specific assay did not amplify DNA from 3 out of 9 strains of S. mutans and 1 out of 3 strains of S. salivarius. There was no amplification with genomic DNA from other bacterial genera (Table 7).

### EXAMPLE 5:

Nucleotide sequencing of DNA fragments. The nucleotide sequence of a portion of the *tuf* genes from a variety of bacterial or fungal species was determined by using the dideoxynucleotide chain termination sequencing method (Sanger *et al.*, 1977, Proc. Natl. Acad. Sci. USA. 74:5463-5467). The sequencing was performed by using an Applied Biosystems automated DNA sequencer (model 373A) with their PRISM™ Sequenase® Terminator Double-stranded DNA Sequencing Kit (Perkin-Elmer Corp.,

- 26 -

Applied Biosystems Division, Foster City, CA). The sequencing strategy does not discriminate tufA and tufB genes because the sequencing primers hybridize efficiently to both bacterial tuf genes. These DNA sequences are shown in the sequence listing (SEQ ID Nos: 118 to 146). The presence of several degenerated nucleotides in the various tuf sequences determined by our group (Table 13) corresponds to sequence variations between tufA and tufB.

Oligonucleotide primers and probes selection. Oligonucleotide probes and amplification primers were selected from the given proprietary DNA fragments or database sequences using the Oligo™ program and were synthesized with an automated ABI DNA synthesizer (Model 391, Perkin-Elmer Corp., Applied Biosystems Division) using phosphoramidite chemistry.

## **EXAMPLE 6:**

5

10

15

20

25

30

35

Labeling of oligonucleotides for hybridization assays. Each oligonucleotide was 5' end-labeled with y-32P (dATP) by the T4 polynucleotide kinase (Pharmacia) as described earlier. The label could also be non-radioactive.

Specificity test for oligonucleotide probes. All labeled oligonucleotide probes were tested for their specificity by hybridization to DNAs from a variety of bacterial and fungal species selected from Tables 4, 5 and 6 as described earlier. Species-specific or genus-specific probes were those hybridizing only to DNA from the microbial species or genus from which it was isolated. Oligonucleotide probes found to be specific were submitted to ubiquity tests as follows.

Ubiquity test for oligonucleotide probes. Specific oligonucleotide probes were then-used-in-ubiquity/tests-with-strains-of-the-target-species-or-genus-including reference strains and other strains obtained from various countries and which are representative of the diversity within each target species or genus. Chromosomal DNAs from the isolates were transferred onto nylon membranes and hybridized with labeled oligonucleotide probes as described for specificity tests. The batteries of isolates constructed for each target species or genus contain reference ATCC strains as well as a variety of clinical isolates obtained from various sources. Ubiquitous probes were those hybridizing to at least 80% of DNAs from the battery of clinical isolates of the target species or genus.

### **EXAMPLE 7:**

Same as example 6 except that a pool of specific oligonucleotide probes is used for microbial identification (i) to increase sensitivity and assure 100% ubiquity or (ii) to identify simultaneously more than one microbial species and/or genus. Microbial identification could be performed from microbial cultures or directly from any clinical specimen.

10

15

20

30

35

### **EXAMPLE 8:**

Same as example 6 except that bacteria or fungi were detected directly from clinical samples. Any biological sample was loaded directly onto a dot blot apparatus and cells were lysed in situ for bacterial or fungal detection and identification. Blood samples should be heparizined in order to avoid coagulation interfering with their convenient loading on a dot blot apparatus.

### **EXAMPLE 9:**

PCR amplification. The technique of PCR was used to increase the sensitivity and the rapidity of the assays. The sets of primers were tested in PCR assays performed directly from bacterial colonies or from a standardized bacterial suspension (see Example 10) to determine their specificity and ubiquity (Table 7). Examples of specific and ubiquitous PCR primer pairs are listed in Annex VI.

Specificity and ubiquity tests for amplification primers. The specificity of all selected PCR primer pairs was tested against DNAs from a variety of bacterial and fungal species selected from Tables 4, 5 and 6 as described earlier. Primer pairs found specific for each species or genus were then tested for their ubiquity to ensure that each set of primers could amplify at least 90% of DNAs from a battery of isolates of the target species or genus. The batteries of isolates constructed for each species contain reference ATCC strains and various clinical isolates from around the world which are representative of the diversity within each species or genus.

Standard precautions to avoid false positive PCR results should be taken (Kwok and Higuchi, 1989, Nature, 239:237-238). Methods to inactivate PCR amplification products such as the inactivation by uracil-N-glycosylase may be used to control PCR carryover.

## 25 **EXAMPLE 10**:

Amplification directly from bacterial or yeast cultures. PCR assays were performed either directly from a bacterial colony or from a bacterial suspension, the latter being adjusted to a standard McFarland 0.5 (corresponds to approximately 1.5 x 10° bacteria/mL). In the case of direct amplification from a colony, a portion of a colony was transferred using a plastic rod directly into a 20  $\mu$ L PCR reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM MgCl<sub>2</sub>, 0.4  $\mu$ M of each primer, 200  $\mu$ M of each of the four dNTPs and 0.5 unit of Taq DNA polymerase (Promega) combined with the TaqStart<sup>TM</sup> antibody (Clontech Laboratories Inc.). For the bacterial suspension, 1  $\mu$ L of the cell suspension was added to 19  $\mu$ L of the same PCR reaction mixture. For the identification from yeast cultures, 1  $\mu$ L of a standard McFarland 1.0 (corresponds to approximately 3.0 x 10° bacteria/mL) concentrated 100 times by centrifugation was added directly to the PCR reaction. This concentration step for yeast cells was performed because a McFarland 0.5 for yeast cells has approximately 200 times fewer cells than a McFarland 0.5 for bacterial cells.

PCR reactions were then subjected to thermal cycling (3 min at 95°C followed by 30 cycles of 1 second at 95°C for the denaturation step and 30 seconds at 55°C for the annealing-extension step) using a PTC-200 thermal cycler. PCR amplification products were then analyzed by standard agarose gel (2%) electrophoresis. Amplification products were visualized in agarose gels containing 0.25  $\mu$ g/mL of ethidium bromide under UV at 254 nm. The entire PCR assay can be completed in approximately one hour.

Primer sequences derived from highly conserved regions of the bacterial 16S ribosomal RNA gene were used to provide an internal control for all PCR reactions. Atternatively, the internal control was derived from sequences not found in microorganisms or in the human genome. The internal control was integrated into all amplification reactions to verify the efficiency of the PCR assays and to ensure that significant PCR inhibition was absent. The internal control derived from rRNA was also useful to monitor the efficiency of the bacterial lysis protocols. The internal control and the species-specific or genus-specific amplifications were performed simultaneously in multiplex PCR assays.

### **EXAMPLE 11:**

5

10

15

20

25

30

35

Amplification directly from urine specimens. For PCR amplification performed directly from urine specimens, 1  $\mu$ L of urine was mixed with 4  $\mu$ L of a lysis solution containing 500 mM KCl, 100 mM tris-HCl (pH 9.0), 1% triton X-100. After incubation for at least 15 minutes at room temperature, 1  $\mu$ L of the treated urine specimen was added directly to 19  $\mu$ L of the PCR reaction mixture. The final concentration of the PCR reagents was-50-mM-KCl<sub>2</sub>-10-mM-Tris-(pH-9.0), 0.1%-Triton-X-100;-2:5-mM-MgCl<sub>2</sub>, 0.4  $\mu$ M of each primer, 200  $\mu$ M of each of the four dNTPs. In addition, each 20  $\mu$ L reaction contained 0.5 unit of Taq DNA polymerase (Promega) combined with the TaqStart<sup>TM</sup> antibody (Clontech Laboratories Inc.).

Strategies for the internal control, PCR amplification and agarose gel detection of the amplicons are as previously described in example 10.

EXAMPLE 12:

<u>Detection of antibiotic resistance genes</u>. The presence of specific antibiotic resistance genes which are frequently encountered and clinically relevant is identified using the PCR amplification or hybridization protocols described previously. Specific oligonucleotides used as a basis for the DNA-based tests are selected from the antibiotic resistance gene sequences. These tests, which allow the rapid evaluation of bacterial resistance to antimicrobial agents, can be performed either directly from clinical specimens, from a standardized bacterial suspension or from a bacterial colony and should complement diagnostic tests for the universal detection of bacteria as well as for the species-specific and genus-specific microbial detection and identification.

10

15

20

25

30

35

## **EXAMPLE 13:**

Same as examples 10 and 11 except that assays were performed by multiplex PCR (i.e. using several pairs of primers in a single PCR reaction) to reach an ubiquity of 100% for the specific targeted pathogen(s). For more heterogeneous microbial species or genus, a combination of PCR primer pairs may be required to detect and identify all representatives of the target species or genus.

Multiplex PCR assays could also be used to (i) detect simultaneously several microbial species and/or genera or, alternatively, (ii) to simultaneously detect and identify bacterial and/or fungal pathogens and detect specific antibiotic resistance genes either directly from a clinical specimen or from bacterial cultures.

For these applications, amplicon detection methods should be adapted to differentiate the various amplicons produced. Standard agarose gel electrophoresis could be used because it discriminates the amplicons based on their sizes. Another useful strategy for this purpose would be detection using a variety of fluorescent dyes emitting at different wavelengths. The fluorescent dyes can be each coupled with a specific oligonucleotide linked to a fluorescence quencher which is degraded during amplification to release the fluorescent dyes (e.g. TaqMan™, Perkin Elmer).

### **EXAMPLE 14:**

Detection of amplification products. The person skilled in the art will appreciate that alternatives other than standard agarose gel electrophoresis (Example 10) may be used for the revelation of amplification products. Such methods may be based on fluorescence polarization or on the detection of fluorescence after amplification (e.g. Amplisensor™, Biotronics; TaqMan™, Perkin-Elmer Corp.) or other labels such as biotin (SHARP Signal™ system, Digene Diagnostics). These methods are quantitative and may be automated. One of the amplification primers or an internal oligonucleotide probe specific to the amplicon(s) derived from the species-specific, genus-specific or universal DNA fragments is coupled with the fluorescent dyes or with any other label. Methods based on the detection of fluorescence are particularly suitable for diagnostic tests since they are rapid and flexible as fluorescent dyes emitting at different wavelengths are available.

### **EXAMPLE 15:**

Species-specific, genus-specific, universal and antibiotic resistance gene amplification primers can be used in other rapid amplification procedures such as the ligase chain reaction (LCR), transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), cycling probe technology (CPT) and branched DNA (bDNA) or any other methods to increase the sensitivity of the test. Amplifications can be performed from isolated bacterial cultures or directly from any clinical specimen. The scope of this invention is therefore not limited to the use of the

10

15

20

25

30

35

DNA sequences from the enclosed Sequence Listing for PCR only but rather includes the use of any procedures to specifically detect bacterial DNA and which may be used to increase rapidity and sensitivity of the tests.

### **EXAMPLE 16:**

A test kit would contain sets of probes specific for each microbial species or genus as well as a set of universal probes. The kit is provided in the form of test components, consisting of the set of universal probes labeled with non-radioactive labels as well as labeled species- or genus-specific probes for the detection of each pathogen of interest in specific types of clinical samples. The kit will also include test reagents necessary to perform the pre-hybridization, hybridization, washing steps and hybrid detection. Finally, test components for the detection of known antibiotic resistance genes (or derivatives therefrom) will be included. Of course, the kit will include standard samples to be used as negative and positive controls for each hybridization test.

Components to be included in the kits will be adapted to each specimen type and to detect pathogens commonly encountered in that type of specimen. Reagents for the universal detection of bacteria will also be included. Based on the sites of infection, the following kits for the specific detection of pathogens may be developed:

- A kit for the universal detection of bacterial or fungal pathogens from all clinical specimens which contains sets of probes specific for highly conserved regions of the microbial genomes.
- A kit for the detection of microbial pathogens retrieved from urine samples, which contains 5 specific test components (sets of probes for the detection of Enterococcus faecium, Enteroccus species, Staphylococcus saprophyticus, Staphylococcus species and Candida albicans).
- A kit for the detection of respiratory pathogens which contains 3 specific test components (sets of probes for the detection of Staphylococcus species, Enterococcus species and Candida albicans).
- A kit for the detection of pathogens retrieved from blood samples, which contains 10 specific test components (sets of probes for the detection of Streptococcus species, Streptococcus agalactiae, Staphylococcus species, Staphylococcus saprophyticus, Enterococcus species, Enterococcus faecium, Neisseria species, Neisseria meningitidis, Listeria monocytogenes and Candida albicans). This kit can also be applied for direct detection and identification from blood cultures.
- A kit for the detection of pathogens causing meningitis, which contains 5 specific test components (sets of probes for the detection of Streptococcus species, Listeria monocytogenes, Neisseria meningitidis, Neisseria species and Staphylococcus species).

10

15

20

25

30

35

- A kit for the detection of clinically important antibiotic resistance genes which contains sets of probes for the specific detection of at least one of the 26 following genes associated with antibiotic resistance:  $bla_{non}$ ,  $bla_{noh}$ ,  $bla_{obn}$ 

 Other kits adapted for the detection of pathogens from skin, abdominal wound or any other clinically relevant infections may also be developed.

## **EXAMPLE 17:**

Same as example 16 except that the test kits contain all reagents and controls to perform DNA amplification assays. Diagnostic kits will be adapted for amplification by PCR (or other amplification methods) performed directly either from clinical specimens or from microbial cultures. Components required for (i) universal bacterial detection, (ii) species-specific and genus-specific bacterial and/or fungal detection and identification and (iii) detection of antibiotic resistance genes will be included.

Amplification assays could be performed either in tubes or in microtitration plates having multiple wells. For assays in plates, the wells will contain the specific amplification primers and control DNAs and the detection of amplification products will be automated. Reagents and amplification primers for universal bacterial detection will be included in kits for tests performed directly from clinical specimens. Components required for species-specific and genus-specific bacterial and/or fungal detection and identification as well as for the simultaneous antibiotic resistance genes detection will be included in kits for testing directly from bacterial or fungal cultures as well as in kits for testing directly from any type of clinical specimen.

The kits will be adapted for use with each type of specimen as described in example 16 for hybridization-based diagnostic kits.

### **EXAMPLE 18:**

It is understood that the use of the probes and amplification primers described in this invention for bacterial and/or fungal detection and identification is not limited to clinical microbiology applications. In fact, we feel that other sectors could also benefit from these new technologies. For example, these tests could be used by industries for quality control of food, water, air, pharmaceutical products or other products requiring microbiological control. These tests could also be applied to detect and identify bacteria or fungi in biological samples from organisms other than humans (e.g. other primates, birds, plants, mammals, farm animals, livestock and others). These diagnostic tools could also be very useful for research purposes including clinical trials and epidemiological studies.

This invention has been described herein above, and it is readily apparent that modifications can be made thereto without departing from the spirit of this invention. These modifications are under the scope of this invention, as defined in the appended claims.

Table 1. Distribution (%) of nosocomial pathogens for various human infections in USA (1990-1992)¹.

	Pathogen	UTI <sup>2</sup>	SSI³	BSI <sup>4</sup>	Pneumonia	CSF⁵
5	Escherichia coli	27	9	5	4	2
	Staphylococcus aureus	2	21	17	21	2
	Staphylococcus epidermidis	2	6	20	0	1
	Enterococcus faecalis	16	12	9	2	0
	Enterococcus faecium	1	1	0	0	0
10	Pseudomonas aeruginosa	12	9	3	18	0
	Klebsiella pneumoniae	7	3	4	9	0
	Proteus mirabilis	5	3	1	2	0
	Streptococcus pneumoniae	0	0	3	1	18
	Group B Streptococci	1	1	2	1	6
15	Other Streptococci	3	5	2	1	3
	Haemophilus influenzae	0	0	0	6	3 45
	Neisseria meningitidis	0	0	0	0	45 14
	Listeria monocytogenes	0	0	0	0	3
	Other Enterococci	1	1	0	0	0
20	Other Staphylococci	2	•	8	13	20
The page as respect	Candida albicans	9	3	5	5	
	Other Candida	2		1	3	.0.
	Enterobacter spp.	5	7	4	12	10
	Acinetobacter spp.	1	1	2	4	2
25	Citrobacter spp.	2	1	1	4	2
	Serratia marcescens	1	1	1	2	0
	Other Klebsiella	1	1	1	3	7
	Others	0	6	4	2	7
		<del>-</del>		4	5	0

<sup>30</sup> ¹ Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, Clin. Microbiol. Rev., 6:428-442).

<sup>&</sup>lt;sup>2</sup> Urinary tract infection.

Surgical site infection.

<sup>4</sup> Bloodstream infection.

<sup>35 &</sup>lt;sup>5</sup> Cerebrospinal fluid.

Tabl 2. Distribution (%) of bloodstream infection pathogens in Quebec (1995), Canada (1992), UK (1969-1988) and USA (1990-1992).

5	Organism	Quebec <sup>1</sup>	Canada <sup>2</sup>	UK³		USA <sup>4</sup>
J	O,ga.no.n			Community- acquired	Hospital- acquired	Hospital- acquired
	E. coli	15.6	53.8	24.8	20.3	5.0
	S. epidermidis and other CoNS <sup>5</sup>	25.8	NI <sub>e</sub>	0.5	7.2	31.0
0	S. aureus	9.6	NI	9.7	19.4	16.0
•	S. pneumoniae	6.3	NI	22.5	2.2	NR <sup>7</sup>
	E. faecalis	3.0	NI	1.0	4.2	NR
	E. faecium	2.6	NI	0.2	0.5	NR
	Enterococcus	NR	NI	NR	NR	9.0
15	spp.	4.5	NR	3.4	0.4	NR
	H. influenzae P. aeruginosa	1.5 1.5	8.2	1.0	8.2	3.0
	K. pneumoniae	3.0	11.2	3.0	9.2	4.0
	P. mirabilis	NR	3.9	2.8	5.3	1.0
20	S. pyogenes	NR	NI	1.9	0.9	NR
1.000	Enterobacter spp.	4.1	5.5	0.5	2.3	4.0
	Candida spp.	8.5	NI	NR	1.0	8.0
	Others	18.5	17.48	28.7	18.9	19.0

- 25 ¹ Data obtained for 270 isolates collected at the Centre Hospitalier de l'Université Laval (CHUL) during a 5 month period (May to October 1995).
  - <sup>2</sup> Data from 10 hospitals throughout Canada representing 941 gram-negative bacterial isolates. (Chamberland et al., 1992, Clin. Infect. Dis., 15:615-628).
  - Data from a 20-year study (1969-1988) for nearly 4000 isolates (Eykyn et al., 1990, J. Antimicrob. Chemother., Suppl. C, 25:41-58).
  - Data recorded by the National Nosocomial Infections Surveillance (NNIS) from 80 hospitals (Emori and Gaynes, 1993, Clin. Microbiol. Rev., 6:428-442).
  - 5 Coagulase-negative staphylococci.
  - <sup>6</sup> NI, not included. This survey included only gram-negative species.
- 35 7 NR, incidence not reported for these species or genera.
  - <sup>8</sup> In this case, 17.4 stands for other gram-negative bacterial species.

Table 3. Distribution of positive and negative clinical specimens tested at the microbiology laboratory of the CHUL (February 1994 – January 1995).

5	Clinical specimens and/or sites	No. of samples tested (%)	% of positive specimens	% of negative specimens
	Urine	17,981 (54.5)	19.4	80.6
	Blood culture/marrow	10,010 (30.4)	6.9	93.1
	Sputum	1,266 (3.8)	68.4	31.6
	Superficial pus	1,136 (3.5)	72.3	27.7
10	Cerebrospinal fluid	553 (1.7)	1.0	99.0
	Synovial fluid	523 (1.6)	2.7	97.3
	Respiratory tract	502 (1.5)	56.6	43.4
	Deep pus	473 (1.4)	56.8	43.2
	Ears	289 (0.9)	47.1	52.9
5	Pleural and pericardial fluid	132 (0.4)	1.0	99.0
	Peritoneal fluid	101(0.3)	28.6	71.4
	Total:	32,966 (100.0)	20.0	80.0

Tabl 4. Gram-n gativ bacterial species (90) used to test the specificity f PCR primers and DNA prob s (continu s on next page).

	Bacterial species	Number of reference strains	Bacterial species	Number of reference strains
		tested		tested*
5	Acinetobacter baumannii	1	Moraxella phenylpyruvica	•
	Acinetobacter Iwoffii	3	Morganella morganii	1
	Actinobacillus lignieresii	1	Neisseria animalis	1
	Alcaligenes faecalis	1	Neisseria canis	1
	Alcaligenes odorans	1	Neisseria caviae	1
10	Alcaligenes xylosoxydans		Neisseria cinerea	1
	subsp. denitrificans	1	Neisseria cuniculi	1
	Bacteroides distasonis	1	Neisseria elongata subsp. elongata	1
	Bacteroides fragilis	1	Neisseria elongata subsp. glycoytica	1
	Bacteroides ovatus	1	Neisseria flavescens	1
15	Bacteroides	1	Neisseria flavescens	1
	thetaiotaomicron		Branham	
	Bacteroides vulgatus	1	Neisseria gonorrhoeae	. 18
	Bordetella bronchiseptica	1	Neisseria lactamica	1
	Bordetella parapertussis	1	Neisseria meningitidis	4
20	Bordetella pertussis	2	Neisseria mucosa	2
	Burkholderia cepacia	1	Neisseria polysaccharea	1
	Citrobacter amalonaticus	1	Neisseria sicca	3
	Citrobacter diversus subsp. koseri	2	Neisseria subflava	3
25	Citrobacter freundii	1	Neisseria weaveri	1
	Comamonas acidovorans	1	Ochrobactrum antropi	1
	Enterobacter aerogenes	1	Pasteurella aerogenes	1
	Enterobacter	1	Pasteurella multocida	1
	agglomerans		Describe molonine conice	, 1
30	Enterobacter cloacae	1	Prevotella melaninogenica	3
	Escherichia coli	9	Proteus mirabilis	1
	Escherichia fergusonii	1	Proteus vulgaris	

	Bacterial species	Number of	Bact rial species	Number of
		reference	Saot Hai species	refer nce
		strains		strains
		tested <sup>a</sup>		tested*
	Escherichia hermannii	1	Providencia alcalifaciens	1
	Escherichia vulneris	1	Providencia rettgeri	1
	Flavobacterium	1	Providencia rustigianii	1
	meningosepticum		a denganii	'
5	Flavobacterium	1	Providencia stuartii	1
	indologenes		The state of	•
	Flavobacterium odoratum	1	Pseudomonas aeruginosa	14
	Fusobacterium	2	Pseudomonas fluorescens	2
	necrophorum		The state of the s	2
10	Gardnerella vaginalis	1	Pseudomonas stutzeri	1
	Haemophilus	1	Salmonella arizonae	1
	haemolyticus			•
	Haemophilus influenzae	12	Salmonella choleraesuis	1
	Haemophilus	1	Salmonella gallinarum	1
15	parahaemolyticus		•	
	Haemophilus	2	Salmonella typhimurium	3
	parainfluenzae		• •	-
			Serratia liquefaciens	1
	Kingella indologenes	1	Serratia marcescens	1
20	subsp. suttonella			
	Kingella kingae	1	Shewanella putida	1
	Klebsiella ornithinolytica	1	Shigella boydii	1
	Klebsiella oxytoca	1	Shigella dysenteriae	1
	Klebsiella pneumoniae	8	Shigella flexneri	1
25	Moraxella atlantae	1	Shigella sonnei	1.6
	Moraxella catarrhalis	5	Stenotrophomonas	1
			maltophilia	•
	Moraxella lacunata	1	Yersinia enterocolitica	1
	Moraxella osloensis	1		•

30 Most reference strains were obtained from the American Type Culture Collection (ATCC). The other reference strains were obtained from (i) the Laboratoire de Santé Publique du Québec (LSPQ), (ii) the Center for Disease Control and Prevention (CDC) and (iii) the National Culture Type Collection (NCTC).

Tabl 5. Gram-positive bacterial species (97) used to t st th sp cificity f PCR primers and DNA probes (continues on n xt pag ).

	Bacterial species	Number of	Bacterial species	Number of reference
		reference		
		strains		strains
		testeda		testeda
	Abiotrophia adiacens	1	Micrococcus kristinae	1,52
	Abiotrophia defectiva	1	Micrococcus luteus	1
	Actinomyces israelii	1	Micrococcus lylae	1
	Clostridium perfringens	1	Micrococcus roseus	1
	Corynebacterium accolens	1	Micrococcus varians	1
	Corynebacterium	1	Peptococcus niger	1
	aguaticum			
	Corynebacterium bovis	1	Peptostreptococcus	1
			anaerobius	
	Corynebacterium cervicis	1	Peptostreptococcus	1 -
	22.,		asaccharolyticus	
	Corynebacterium	6	Staphylococcus aureus	10
;	diphteriae			
•	Corynebacterium	1	Staphylococcus auricularis	1
	flavescens			
	Corynebacterium	6	Staphylococcus capitis	1
	genitalium		subsp. urealyticus	
)	Corynebacterium jeikeium	1	Staphylococcus cohnii	1
•	Corynebacterium kutcheri	1	Staphylococcus epidermidis	
	Corynebacterium	1	Staphylococcus	2
	matruchotii		haemolyticus	
	Corynebacterium	1	Staphylococcus hominis	2
5	minutissimum			
5	Corynebacterium	1	Staphylococcus	1
	mycetoides		lugdunensis	
	Corynebacterium	4	Staphylococcus	3
	pseudodiphtheriticum		saprophyticus	
0	Corynebacterium	6	Staphylococcus schleiferi	1
ou	pseudogenitalium	_		
	Corynebacterium renale	1	Staphylococcus sciuri	1
	Corynebacterium striatun		Staphylococcus simulans	1
	Corynebacterium ulceran		Staphylococcus warneri	1

.... 1011 - 444

	Bacterial species	Number of	f Bacterial species	Number of
		reference		reference
		strains		strains
		tested		tested*
	Corynebacterium	1	Staphylococcus xylosus	1
	urealyticum		•	•
	Corynebacterium xerosis	1	Streptococcus agalactiae	6
	Enterococcus avium	1	Streptococcus anginosus	2
5	Enterococcus	1	Streptococcus bovis	2
	casseliflavus		,	2
	Enterococcus cecorum	1.	Streptococcus constellatus	1
	Enterococcus dispar	1	Streptococcus crista	1
	Enterococcus durans	1	Streptococcus dysgalactiae	1
10	Enterococcus faecalis	6	Streptococcus equi	1
	Enterococcus faecium	3	Streptococcus gordonii	1
	Enterococcus flavescens	1	Group C Streptococci	•
	Enterococcus gallinarum	3	Group D Streptococci	1 1
	Enterococcus hirae	1	Group E Streptococci	1
15	Enterococcus mundtii	1	Group F Streptococci	,
	Enterococcus	1	Group G Streptococci	1
	pseudoavium		arap a di optococci	
	Enterococcus raffinosus	1	Streptococcus intermedius	4
	Enterococcus	1	Streptococcus mitis	1
20	saccharolyticus			2
	Enterococcus solitarius	1	Streptococcus mutans	Thorne the co
	Eubacterium lentum		Streptococcus oralis	1
	Gemella haemolysans		Streptococcus parasanguis	1
	Gemella morbillorum		Streptococcus pneumoniae	1
25	Lactobacillus acidophilus		Streptococcus pyogenes	6
	Listeria innocua		Streptococcus salivarius	3
	Listeria ivanovii		Streptococcus sanguis	2
	Listeria grayi		Streptococcus sobrinus	2
	Listeria monocytogenes		Streptococcus suis	1
30	Listeria murrayi		Streptococcus suis Streptococcus uberis	1
	Listeria seeligeri		Streptococcus uneris	1
	Listeria welshimeri	1	on optiococcus vestibularis	1

<sup>&</sup>lt;sup>a</sup> Most reference strains were obtained from the American Type Culture Collection 35 (ATCC). The other reference strains were obtained from (i) the Laboratoire de Santé Publique du Québec (LSPQ), (ii) the Center for Disease Control and Prevention (CDC) and (iii) th National Culture Type Collection (NCTC).

Tabl 6. Fungal sp cies (12) used to test the specificity of PCR primers and DNA probes.

	Fungal species	Number of reference		
5		strains tested <sup>a</sup>		
	Candida albicans	12		
	Candida glabrata	1		
	Candida guilliermondii	1		
	Candida kefyr	3		
10	Candida krusei	2		
	Candida lusitaniae	1		
	Candida parapsilosis	2		
	Candida tropicalis	3		
	Rhodotorula glutinis	1		
15	Rhodotorula minuta	1		
	Rhodotorula rubra	1		
	Saccharomyces cerevisiae	1		

Most reference strains were obtained from (i) the American Type Culture Collection (ATCC) and (ii) the Laboratoire de Santé Publique du Québec (LSPQ).

Table 7. PCR assays developed for several clinically important bacterial and fungal pathogens (continues on next pag.).

	Organism	Primer Pair <sup>a</sup>	Amplicon	Ubiquity <sup>b</sup>	DNA ampl	ification from
		SEQ ID NO	size (bp)		culturec	specimens⁴
	Enterococcus faecium	1-2	216	79/80	+	+
5	Listeria monocytogenes	3-4	130	164/168°	+	+
	Neisseria meningitidis	5-6	177	258/258	+	+
	Staphylococcus saprophyticus	7-8	149	245/260	+	NT
10	Streptococcus agalactiae	9-10	154	29/29	+	+
	Candida albicans	11-12	149	88/88	+	NT
	Enterococcus	13-14	112	87/87	+	NT
	spp. (11 species) <sup>f</sup>					
	Neisseria spp.	15-16	103	321/321	+	+
15	(12 species)f					
	Staphylococcus spp.	17-18	192	13/14	+	NT .
	(14 species)					
	4.5	19-20	221	13/14	+	NT
(10.0)	Streptococcus spp.	21-22	153	210/2149	· ~ <b>+</b> ~~	
20	(22 species)f					
	Universal detectionh	23-24	309	104/ 116 <sup>i</sup>	+	+
	(95 species) <sup>i</sup>					

- All primer pairs are specific in PCR assays since no amplification was observed
   with DNA from the bacterial and fungal species other than the species of interest listed in Tables 4. 5 and 6.
  - b Ubiquity was tested by using reference strains as well as strains from throughout the world, which are representatite of the diversity within each target species or genus.
- 30 ° For all primer pairs, PCR amplifications performed directly from a standardized microbial suspension (MacFarland) or from a colony were all specific and ubiquitous.
  - d PCR assays performed directly from blood cultures, urine specimens or

15

cerebrospinal fluid. NT, not tested.

- The four L. monocytogenes strains undetected are not clinical isolates. These strains were isolated from food and are not associated with a human infection.
- The bacterial species tested include all those clinically relevant for each genus (Tables 4 and 5). All of these species were efficiently amplified by their respective genus-specific PCR assay, except for the Staphylococcus-specific assay, which does not amplify S. sciuri.
  - The Streptococcus-specific PCR assay did not amplify 3 out of 9 strains of S. mutans and 1 out of 3 strains of S. salivarius.
- 10 h The primers selected for universal bacterial detection do not amplify DNA of non-bacterial origin, including human and other types of eukaryotic genomic DNA.
  - For the universal amplification, the 95 bacterial species tested represent the most clinically important bacterial species listed in Tables 4 and 5. The 12 strains not amplified are representatives of genera *Corynebacterium* (11 species) and *Stenotrophomonas* (1 species).

Table 8. Target genes for the various genus-specific, species-specific and universal amplification assays.

Microorganisms	Gene	Protein encoded
Candida albicans	tuf	translation elongation factor EF-Tu
Enterococcus faecium	ddl	D-alanine:D-alanine ligase
Listeria monocytogenes	actA	actin-assembly inducing protein
Neisseria meningitidis	отр	outer membrane protein
Streptococcus agalactia	e cAMP	cAMP factor
Staphylococcus	unknown	unknown
saprophyticus		
Enterococcus spp.	tuf	translation elongation factor EF-1
Neisseria spp.	asd	ASA-dehydrogenase
Staphylococcus spp.	tuf	translation elongation factor EF-1
Streptococcus spp.	recA	RecA protein
Universal detection	tuf	translation elongation factor EF-1

Tabl 9. Antibiotic resistanc genes selected for diagnostic purpos s.

	Genes	SEQ I	D NOs	Antibiotics	Bacteriaª
		selected primers	originating fragment	-	
5	bla <sub>oxa</sub>	49-50	110	β-lactams	Enterobacteriaceae, Pseudomonadaceae
	blaZ	51-52	111	β-lactams	Enterococcus spp.
	aac6'-lla	61-64	112	Aminoglycosides	Pseudomonadaceae
	ermA	91-92	113	Macrolides	Staphylococcus spp.
10	ermB	93-94	114	Macrolides	Staphylococcus spp.
	ermC	95-96	115	Macrolides	Staphylococcus spp.
	vanB	71-74	116	Vancomycin	Enterococcus spp.
	vanC	75-76	117	Vancomycin	Enterococcus spp.
15	aad(6')	173-174		Streptomycin	Enterococcus spp.

<sup>&</sup>lt;sup>a</sup> Bacteria having high incidence for the specified antibiotic resistance genes. The presence of these antibiotic resistance genes in other bacteria is not excluded.

Tabl 10. Antibiotic resistance genes from our co-pending US (N.S. 08/526840) and PCT (PCT/CA/95/00528) pat nt applications for which we have selected PCR primer pairs.

5	Genes	SEQ ID NOs	Antibiotics	Bacteria <sup>a</sup>
-		of selected primers		
	bla <sub>tem</sub>	37-40	β-lactams	Enterobacteriaceae,
	10111			Pseudomonadaceae,
				Haemophilus spp.,
				Neisseria spp.
	blamb	45-48	β-lactams	Haemophilus spp.,
	Diamb			Pasteurella spp.
_	blastv	41-44	β-lactams	Klebsiella spp.
0	Didshv			and other
				Enterobacteriaceae
	aadB	53-54	Aminoglycosides	Enterobacteriaceae,
	aacC1	55-56		Pseudomonadaceae
	aacC1	57-58		
5	aacC2 aacC3	59-60		
5	aacA4	65-66		
	mecA	97-98	B-lactams	Staphylococcus spp
	vanA	67-70	Vancomycin	Enterococcus spp.
	satA	81-82	Macrolides	Enterococcus spp.
0.	aac(6')-aph(2")	83-86	Aminoglycosides	Enterococcus spp.,
.0	aac(o) up(= )			Staphylococcus spp
	vat	87-88	Macrolides	Staphylococcus spp
	vga	89-90	Macrolides	Staphylococcus spp
	msrA	77-80	Erythromycin	Staphylococcus spp
	int	99-102	β-lactams,	Enterobacteriaceae
25			trimethoprim,	
	sul	103-106	aminoglycosides,	Pseudomonadacea
	Ju.		antiseptic,	
			chloramphenicol	

Bacteria having high incidence for the specified antibiotic resistance genes. The presence of these antibiotic resistance genes in other bacteria is not excluded.

Table 11. Correlation between disk diffusion and PCR amplification of antibiotic resistance genes in *Staphylococcus* species\*.

				5		
-				Disk d	iffusion (Kirby-E	lauer) <sup>b</sup>
5	Antibiotic	Phenotype	PCR	Resistant	Intermediate	Sensitive
	Penicillin	blaZ	+	165	0	0
			-	0	0	31
	Oxacillin	mecA	+	51	11	4
40			-	2	0	128
10	Gentamycin	aac(6')aph(2")	+	24	18	6
			-	0	0	148
	Erythromycin	ermA	+	15	0	0
		ermB	+	0	0	0
		ermC	+	43	0	0
15		msrA	+	4	0	0
			-	0	1	136

- The Staphylococcus strains studied include S. aureus (82 strains), S. epidermidis (83 strains), S. hominis (2 strains), S. capitis (3 strains), S. haemolyticus (9 strains), S. simulans (12 strains) and S. warneri (5 strains), for a total of 196 strains.
- Susceptibility testing was performed by the method of Kirby-Bauer according to the protocol reccommended by the National Committee of Clinical Laboratory Standards (NCCLS).

Table 12. Correlation between disk diffusion profiles and PCR amplification of antibiotic resistance genes in *Enteroc ccus* speci s<sup>a</sup>.

			Disk diffusion	(Kirby-Bauer) <sup>b</sup>
Antibiotic	Phenotype	PCR	Resistant	Sensitive
	blaZ	+	0	2
Ampicillin				
		-	1	30
Gentamycin	aac(6')aph(2'')	+	51	1
		-	3	38
Streptomycin	aad(6')	+	26	15
		-	6	27
Vancomycin	vanA	+	36	0
	vanB	+	26	0
		_	0	40

20

- The Enterococcus strains studied include E. faecalis (33 strains) and E. faecium (69 strains), for a total of 102 strains.
- Susceptibility testing was performed by the method of Kirby-Bauer according to the protocol reccommended by the National Committee of Clinical Laboratory Standards (NCCLS).

Tabl 13. Origin of *tuf* sequ nces in the Sequence Listing (continues on next page).

SEQ ID NO		
SEQ ID NO	Bacterial or fungal species	Source
118	Abiotrophia adiacens	This patent
119	Abiotrophia defectiva	This patent
120	Candida albicans	This patent
121	Candida glabrata	This patent
122	Candida krusei	This patent
123	Candida parapsilosis	This patent
124	Candida tropicalis	This patent
125	Corynebacterium accolens	This patent
126	Corynebacterium diphteriae	This patent
127	Corynebacterium genitalium	This patent
128	Corynebacterium jeikeium	This patent
129	Corynebacterium	This patent
	pseudotuberculosis	
130	Corynebacterium striatum	This patent
131	Enterococcus avium	This patent
132	Enterococcus faecalis	This patent
133	Enterococcus faecium	This patent
134	Enterococcus gallinarum	This patent
135	Gardnerella vaginalis	This patent
136	Listeria innocua	This patent
137	Listeria ivanovii	This patent
138	Listeria monocytogenes	This patent
139	Listeria seeligeri	This patent
140	Staphylococcus aureus	This patent
141	Staphylococcus epidermidis	This patent
142	Staphylococcus saprophyticus	This patent
143	Staphylococcus simulans	This patent
144	Streptococcus agalactiae	This patent
145	Streptococcus pneumoniae	This patent
	118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143	118 Abiotrophia adiacens 119 Abiotrophia defectiva 120 Candida albicans 121 Candida glabrata 122 Candida krusei 123 Candida parapsilosis 124 Candida tropicalis 125 Corynebacterium accolens 126 Corynebacterium diphteriae 127 Corynebacterium genitalium 128 Corynebacterium genitalium 129 Corynebacterium jeikeium 129 Corynebacterium striatum 130 Corynebacterium striatum 131 Enterococcus avium 132 Enterococcus faecalis 133 Enterococcus faecium 134 Enterococcus gallinarum 135 Gardnerella vaginalis 136 Listeria innocua 137 Listeria ivanovii 138 Listeria monocytogenes 139 Listeria seeligeri 140 Staphylococcus aureus 141 Staphylococcus saprophyticus 143 Staphylococcus simulans 144 Streptococcus agalactiae

-	SEQ ID NO	Bacterial or fungal species	Source
-	146	Streptococcus salivarius	This patent
	147	Agrobacterium tumefaciens	Database
	148	Bacillus subtilis	Database
	149	Bacteroides fragilis	Database
5	150	Borrelia burgdorferi	Database
	151	Brevibacterium linens	Database
	152	Burkholderia cepacia	Database
	153	Chlamydia trachomatis	Database
	154	Escherichia coli	Database
10	155	Fibrobacter succinogenes	Database
	156	Flavobacterium ferrugineum	Database
	157	Haemophilus influenzae	Database
	158	Helicobacter pylori	Database
	159	Micrococcus luteus	Database
15	160	Mycobacterium tuberculosis	Database
	161	Mycoplasma genitalium	Database
	162	Neisseria gonorrhoeae	Database
	163	Rickettsia prowazekii	Database
	164	Salmonella typhimurium	Database
20	165	Shewanella putida	Database
	166	Stigmatella aurantiaca	Database
	167	Streptococcus pyogenes	Database
	168	Thiobacillus cuprinus	Database
	169	Treponema pallidum	Database
25	170	Ureaplasma urealyticum	Database
	171	Wolinella succinogenes	Database

- 48 -

	Annex I:	Strategy f r the selectin from tuf sequences of the universal amplification
		primers (continues on pages 49 to 51).
		4
		491 517776 802 NO
ιΩ	Abiotrophia	Ch <u>actgiaac iggigitigaa aigti</u> ccha <u>alggi aaigcciggi</u> gairacgira
	adiacens	
	Abiotrophia	CTACCGTTAC CGGTGTTGAA ATGTICCAAAIGGI TATGCCAGGC GACAACGTAC
	defectiva	
	Agrobacterium	CGACTGTIAC CGGCGTTGAA AIGTICCAAAIGGI IATGCCTGGC GACAACGTCA
10	tumefaciens	
	Bacillus	CAACTGITAC AGGIGITGAA AIGTICCAAATGGI TATGCCTGGA GATAACACTG
	subtilis	0.5
	Bacteroides	CAGTIGIAAC AGGIGINGAA AIGIICCAAAIGGI AAIGCCGGGI GAIAAGGIBA
	fragilis	
15	Borrelia	CTACTGITAC IGGIGITGAA AIGITCCAAAIGGI TAIGCCIGGI GATAATGITG
	burgdorferi	
	Brevibacterium	CGACTGICAC CGCIAICGAG AIGTICCAGAIGGT CAIGCCCAAC GACACCACA
	linens	
	Burkholderia	CGACCIGCAC GGGCGTIGAA AIGITCCAAAIGGT CAIGCCGGGC GACAAAGGGG
20	cepacia	7.27
	Chlamydia	CCATISTIAC IGGGGIIGAA AIGTICAAGAIGGI CAIGCCTGGG GAIAACGTNG
	trachomatis	707
	Corynebacterium	CCACCGITAC CGGIAICGAG AIGIICCAGAIGGI CAIGCCIGGC GACAAACTICG
	diphteriae	126

BNSDOCID: <WO 9820157A2 1 >

RGGI TATGCCGGGC GACAACGITG	128	IS2	133	IGGI AAIGCCGGGC GACAACAICA	<u>IGGI TACTCCGGGI GACA</u> CG <u>GI</u> CA	ISE INTOCCIGGI GALAACACCA	135 Neget Tchgcchagg Garcacha	157	158 INTECCTOGE GATAATGIGA	AIGGI AAVGCCIGGI GAIAACALIG	AIRGI CAIGCCCGGC GACAACACCG	ANGOT GARACACCA
Corynebacterium CC <u>ACCGNTAC</u> <u>CTCCATCGAG ATGTT</u> CAAG <u>ATGGT TATGCCGGGC GACGACGT</u> TG	CCACCGTIAC ETCENTGAG ATGITCAAGAIGGT TANGCCGGGC GACAACGITG	CARCHIAC AGRICICAA ANGIICCAANGGI AAIGCIIGGI GAIAAGGITO	CAACAGITAC IGGIGITGAA AIGIICCAAAIGGI CAIGCCCGGI GACAACGI.	CTACCTGIAC TGGGGTTGAA AIGTICC NGAIRGGI AATGCCGGGG GACAAGAICA	ACOTEMICAE GOGICTICAA AISTICOAAAIGGE IACTEGGGGGE GACACGGICA	CTACCGITAC AGGIGITGAG ANGITCC NAALGGY TAIGCCIGGI GATAACACA	CCACCGICAC CTCLAIGGAG ACCTICCAAANGGI ICAGGCAGGC GAITGACGCAA	CINCUGIAAG GGGIGTIGAA AIGIICCAAAIGGI BAIGCCAGGG GAIBACAICA	CO <u>SCIGIASC CGGIGIAGAA AIGII</u> TAAA <u>INGGI IAIGCIIGGC</u> GAIBAIGIGA	Thota <u>girac iggagtagra aigit</u> ccba <u>aiggt</u> aaygg <u>ciggi gatbacai</u> tc	CCA <u>CTGTCAC GGGGATGGAG ATGTT</u> CCAG <u>AIGGT CAIGGGGGG GACAACA</u> CG	COACCITCAC COGRETICAR AIGITCCAGANGOT GAIGCCCGGT GACAACACA
Corynebacterium C	genitalium Corynebacterium (	jeikeium Enterococcus	faecalis Enterococcus	faecium Escherichia	coli Fibrobacter	succinogenes Flavobacterium	ferrugineum Gardnerella	vaginalis Haemophilus	influenzae Helicobacter	pylori Listeria	monocytogenes Micrococcus	<pre>luteus S Mycobacterium</pre>

- 50 -

	. 161		162		163		164		165		166		140		141		144		145		167		168		169
	֥		4		ď		at at		ď																
E	- Services	THE PERSON OF TH	W. Carenda Care		GATARTECT		SACRACATO		CHIACATO		OVERWARD OF	0.000	01 70000000	CHILD & & D	917000	THE PERSON NAMED IN	WI TANKATU		ALAACGTGA		HOTO TOWN		ATANTETER		ATARCACCA
TCTACCTGGT		ATGCCCC	4200000	* COMPANY	Washington,	ATGCCCCC	200	000000000000000000000000000000000000000	200000000000000000000000000000000000000	ATGCCGGG		ATGCCTGGT		e Jeensee		TGC CTCG	100 100	, managananan	100000000	Telemon	***************************************	0 0000000000000000000000000000000000000	5 7557775		55557750
.AAATGGT		. AAATGGT		AGATGGT		AGATGGT »		AGATGGT A		AGAIGGI G		AAATGGT A		AAATGGT TA		AAATGGT T		AAATGGT AA		AAATGGT TA		AAATGGT CA		AD TESTINATION	- AND
 ATGITCA.		ATGTTCC		ATGTTCA	y	ATGTTCC	-1	ATGITCC	. v - ~	ATGITCC		ATGTTCC.	- ••	TGTTCC		ATGTTCC.		ATGITCC.	۱,	TGTTCC		TGTTCA		TGTTTA	
TGGAATTGAA		CGGCGTTGAA		AGGTGTAGAA		GGCGTTGAA		GGTGTAGAA		GGGGTGGAG		GGTGTTGAA		GTGTAGAA A		GGTGTTGAA		GTGTTGAA		GIGITGAA		GCGTGGAA		GCATTGAG A	
CAGTIGITAC IGGAATIGAA AIGIICAAAAIGGI ICIACCIGGI Gamaamoomm		CCACCIOTAC CGGCGITGAA AIGITCC BAATGGT AATGCTCCC		CGACTIGIAC AGGIGIAGAA AIGITICA AGARAGA TAMAMAMA		CTACCIGIAC IGGCGINGAA AIGTICC AGAIGGT AANGCHCGGC		CAACGIGIAG IGGIGIAGAA AIGITGC AGAIGGI AAMGGGAAGA GAMAAGA		CGGTCAICAC GGGGGGGG AIGTICC AGATGGT GATGCTGGG		CAACTGITAC AGGIGITGAA AIGIICCAAAIGGI AAIGCCHGCH GATAAAAAG		CAACTGITAC IGGIGIAGAA AIGIICCAAAIGGI IAIGCTIGGC GACAACCTITA		CAGTIGITAC IGGIGITICAA AIGITCCAAAIGGI TATGCCTGG CAMAACC		CAGTIGITAC IGGIGITGAA AIGITCC AAATGGT AARGCTTGCT CATTLE		CIGI <u>IGITAC IGGIGITGAA A</u> ICITCC AAARGSI TARGCTIGG		CCACCIGGAC CGGCGTGGAA AIGITCAAAATGGT CAACCIGCAC		CAGT <u>GGTIAC TGGCA</u> TTGAG ATGTTTA ACATGGT GAAGA	
Mycoplasma	genitalium	Neisseria	gonorrhoeae	Rickettsia	prowazekii	Salmonella	typhimurium	Shewanella	putida	Stigmatella	aurantiaca	Staphylococcus	aureus	Staphylococcus	epidermidis	Streptococcus	agalactiae	Streptococcus	pneumoniae	Streptococcus	pyogenes	Thiobacillus	cuprinus	Тгеропета	pallidum
		٠		s	7		-		10 F	V)	щ	V)	æ	15 8	ø.	Ø	ά	ξ	20 pi	ß	ſď	T.	ວີ	25 Tz	Da

	Ureaplasma	CTGTIGITAC AGGAAIIGAA AIGTITAATTIGGI TAIGCCAGGI GAIGACGITG	AGGAATTGAA	ATCTTA.	ATTIGGE	TATGCCAGGT	<u>gatgacgt</u> tg	170
	urealyticum							į
	Wolinella	CAACCGIAAC IGGCGTIGAG AIGTICCAGAIGGI IAIGCCIGGI GACAACGITA	TGGCGTTGAG	ATGITCC	. AGATGGT	TATGCCTGGT	GACAACGETA	7/7
	succinogenes							
'n	Candida	GIGTIACCAC IGAAGICAAR ICCCIIGAGRAATI GGAAGAAAAI CCAAAATICG	TGAAGTCAAR	TCCGITG	AGRAATI	<u>G</u> GAAGA <u>A</u> AA <u>T</u>	CC <u>rara</u> TCG	120
	albicans							
	Schizo-	GIGICACIAC CORAGICAAG ICIGIIGAGAAGAI IGAGGAGICC CCIAAGIIIG	CGAAGTCAAG	TCIGITG	. AG <u>aagat</u>	<u>T</u> GA <u>G</u> GA <u>G</u> TC <u>C</u>	CCIAAGTITG	
	saccharomyces pombe			~				
	Human	TGACAGGCAT	<u>TGAGATG</u> TTC	CACAAGA	. AG <u>bagg</u> ag	IGAGAIGITC CACAAGAAGAAGGAGCITGCCAIG CCCGGGGAAGG	cccaagaac	
. 01	Selected®	ACIKKIAC	IGGIGIIGAR AIGII	ATGII	ATGGT	ATGGT IATGCCIGGI GAIAAYRI	GAIAAYRI	
	equences			-				
	Selected	SEQ	SEQ ID NO:23			SEQ ID NO: 24b	10: 24 <sup>b</sup>	
	universal			-474				
15	primer	ACIKKIAC	ACIKKIAC IGGIGTIGAR ATGTT	ATGTT	AYR	r ircicció	AYRTT ITCICCIGGC ATIACCAT	
	sednences,:							
							1	

- 51 -

The sequence numbering refers to the E. coli tuf gene fragment. Underlined nucleotides are identical to the selected sequence or match that sequence.

"I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides A, C, G or T. "K", "R" and "Y" designate nucleotide positions which are degenerated. "K" stands for I or G; "R" stands for A or G; "Y" stands for C or I.

20

c for	
specific for	
n primers	
Strategy for the selection from tuf sequences of the amplificati n primers spo	
the	54)
¥	and
sednenčes	continues on pages 53 and 54)
tuf	8 00
from t	ontinue
selection	ocus (c
the s	erocc
for	Rnt
È	the genus R
ō.	

		314		1 0,	348	401			435	SEQ	
	:			e- "						ON CI	
•	5 Bacillus	CGCGACACTG	AAAAACCATT	CATGATGCCA	GTTGA.		ACAA <u>GTT</u> AAA	CGCGAC <u>ACTG AAAAACCATT CAIGAIG</u> CCA GTTGACGCGG ACAA <u>GIT</u> AAA <u>GICGGIGACG AAGTI</u> GAAAI	AAGTIGAAAT	148	
	subtilis			tet							
	Bacteroides	CGCGAIGTIG	ATAAACCTII	CTIGATGCCG	GTAGA.	ACTGG	TGTTAICCAI	CGCGA <u>TGTIG ATAAACCTII CTIGAIG</u> CCG GTAGAACTGG TGTTAICCAI GTAGGTGAIG AAATCCAAAT	TREEDING	9,7	
	fragilis			٠.,			1			C P	
	Burkholderia	CGTGCAGTIG	ACGCCGCGII	CCTGATGCCG	GTGGA.	09099	CATCGIGAAG	CGTGCRGT <u>IG AC</u> GGCG <u>CGII CCIGAIG</u> CCG GTGGACGCGG CATC <u>GIGAAG</u> G <u>TCGGCGAAG AAATCGAAA</u> T	AAATCGaaaT	153	
10	cepacia									1	
	Chlamydia	AGAGAAATIG .	ACAAGCCTTT	CTIAAIGCCT	ATTGA.	cgrag	AATTGTIAAA	agagaa <u>rtig agaagcctti ctlaaig</u> cct aftgacgtgg aatt <u>gtl</u> aaa gtitccgata aagitcagtt	AAGTTCAGTT	153	
	trachomatis									) }	
	Corynebacterium	CGTGAGACCG	ACAAGCCATT	CCICAIGCCI	ATCGA.	CGTGG	CTCCCTGAAG	CGTGAGA <u>CCG ACAAGCCAIT CCTCAIG</u> CCI ATCGACGTGG CTCCC <u>TGAAG GTCAACGAGG ACGTCGAGA</u> T	ACGTCGAGAT	126	
	diphteriae									2	
15	Enterococcus	CGTGATACTG 1	ACAAACCATT	CATGATGCCA	GTCGA.	CGTGG	ACAAGTTCGC	CGTGA <u>TACTG ACAAACCATT CATGATG</u> CCA GTCGACGTGG ACAA <u>GTTCGC GTTGGTGACG AAGTTGAAA</u> T	AAGTTGAAAT	131	
	avium									i i	
	Enterococcus	CGTGATACTG 1	ACAAACCATT	CATGATGCCA	GTCGA.	cerge	TGAAGTTCGC	CGTGA <u>TACTG ACAAACCATT CATGATG</u> CCA GTCGACGTGG TGAA <u>GTTCGC</u> GTTGGTGACG AAGTTGAAAT	AGTTGAAAT	132	
	faecalis									707	
	Enterococcus	CGTGACAACG 2	ACAAACCATT	CATGATGCCA	GTTGA.	CGTGG	ACAAGTTCGC	CGTGAC <u>AACG ACAAACCAIT CAIGAIG</u> CCA GTTGACGTGG ACAAGTICGC GTTGGTGACG AAGTTGAAGT	AGTTGAAGT	133	
20	faecium								10000	733	
	Enterococcus	CGTGATACTG A	CAAACCATT	CATGATGCCA	GTCGA.	.cGTGG	ACAAGTICGC	CGTGA <u>TACTG ACAAACGAIT CAIGAIG</u> CCA GTCGACGTGG ACAAGTICGC GITGGTGAIG AAGTAGAAA	AGTACAAAT	157	
	gallinarum									5	
	Escherichia	CGTGCGATIG ACAAGCCGII CCIGCIGCCG ATCGACGCGG TATCATCAAA GTTGGTGAAA AAATTAAA	CAAGCCGII	CCIGCIGCCG	ATCGA.	. CGCGG	PATCATCAAA	STTGGTGBBC B	Hara a a contract of	į	
	coli							0	THURSTES	154	

- 52 -

Annex II:

CACGA<u>NCTIG ACAAGCGAII CTIGAIG</u>CCA AICGA....CGFGG TAAGC<u>ICCCA AICAACACC</u> C<u>AGII</u>GAGAI

Gardnerella Haemophilus

BNSDOCID: <WO

140 141

165

162 164 142

- 53 138 159 160 161 157 158 cotgaagta<u>g ataaacttt</u> ctrafraga altga...agagg tgaac**tcaa**a <u>grago</u>caag <u>aagtr</u>gaaat CGCGAG<u>alca acaagcott</u> co<u>taara</u>cog gtcga....cgcgg cgtga<u>rcaac gt</u>gaaa<u>cga</u>gg <u>aactt</u>gagat CGTGCGATIG A<u>CCAACCGII</u> CCITCITCCA AICGA...CGAGG TATTAICCGI ACA<u>GGIGAIG AAGIA</u>GAAAI agaga<mark>cici aaaaactii ctigaig</mark>ccg sttga...agagg cgtgaaaa g<u>eaggcga</u>tg <u>aagtggaaaa</u>

cotga<u>ircte acaarccaft caitgais</u>cca sttga...cgtgg acaa<u>gttaaa gtiggtgacg aagt</u>agaagt

CSCGAC<u>larg acargett cotsais</u>ccs atcsa...cscss caccc<u>t</u>saas a<u>tcaactc<u>s</u>s <u>asstcaaa</u>t</u>

monocytogenes

Listeria

pylori

Helicobacter

'n

influenzae vaginalis

Mycobacterium tuberculosis Micrococcus 10 luteus

Mycoplasma genitalium Neisseria 12

gonorrhoeae typhimurium Salmonella

COTGCGATIG ACAGCGII CCIGCIGCCG ATCGA...CGCGG TATCAICAAA GIGGGCGAAG AAGIIGAAAT CGTGG TATTGINGEG ATAGGCGII CTRATECCA ATCGA...CGTGG TATTGINGGC GINGGCGAAG AAGITGAAAT CGTGA<u>ITCIG ACLARCENII CANGANG</u>CCA GITGA...CGTGG TCAAA<u>I</u>CAAA <u>GIIGGIGAAG AAGII</u>GAAAI cstsa<u>ttetg acaancentt catgan</u>geca gittga...cgtgg teaaa<u>teaaa giwggtgaag aagtt</u>gaaat

COTGCCGTG<u>G ACLAACCAIT</u> <u>CCTGCTG</u>CCT AICGA....CGAGG TAICA<u>ICGAG GITGSIGACG AGAIT</u>GAAAI

Shewanella

Staphylococcus 20 putida aureus

Staphylococcus epidermidis

cotga<u>ticts acaaaccatt catgat</u>scca sttga...cstgs tcaaa<u>tcaaa stcssigaas aaat</u>cgarat

25 Staphylococcus

saprophyticus

	144		145		167		170				
									AAGTT		
CGIGAIACIG ACABACCTII ACIICIICCA GIIGACGIGG DACIGIINGE GERAAGA	ST CHACKACE	CGTGACACTG ACABACCATT GCTTCTTCCA GTCGACGTGG TATCGTTBARA	STEP THE TEN	CGCGACACTG ACABACCATT GCTTCTTCCA GTCGACGTGG TACTGGTTCCT CTACTTCCT	STREET WATER		GITANICATE !		GITCGC GITGGTGACG		SEQ ID NO: 14
TACTGTTCGT	200	TATOGRADA	WW 77	Tacteriors		TGTATTABA	WWW TUTO		97116		as
ACGTGG		ACGTGG		ACGTGG		A CGTGG					
GTTG		GTCG		GTCG		ATTG					
ACTICITICA	-	GCITCITCCA	6	GCTTCTTCCA		CTTATTAGCA	٠	CATGATG		· · · · · ·	
ACAAACCTTT		ACAAACCATT		ACAAACCATT		CAAACCATT		TACTG ACAAACCATT		SEQ ID NO: 13	
CGTGATACTG		CGTGACACTG		CGCGACACTG		CGTAG <u>TACTG ACAAACCAIT</u> CTTATTAGCA ATTGA CGTGG TGTATTAAAA		TACTG 2		S	
Streptococcus	agalactiae	Streptococcus	pneumoniae	5 Streptococcus	pyogenes	Ureaplasma	urealyticum	Selected	10 sequences	Selected	genus-specific
-	-	-	7	ro O	14	7	p	υ	10 8	νi	ťη

The sequence numbering refers to the E. faecalis tuf gene fragment. Underlined nucleotides are identical to the selected sequence or match that sequence

- 54 -

AACTIC GICACCAACG CGAAC

This sequence is the reverse complement of the above tuf sequence. 20 4

The above primers also amplify tuf sequences from Abiotrophia species; this genus has recently been related to the Enterococcus genus by 168 rRNA analysis. NOTE:

primer 15 sequences:

TACTG ACAAACCATT CATGATG

BNSDOCID: <WO 9820157A2 1 >

135

CGGTCGTGTT GAGCGGGGA AGCTCCCAAT CAA.....TGG CTGCTCCAGG ITCTGIGACT CCACACACA CGGTCGTGTA GAACGCGGTA TCATCAAAGT TGG.....TGG CTAAGCCGGG CACCATCAAG CCGCACACCA

> Gardnerella Escherichia

20 coli

vaginalis

133 154

AGGI<u>CGIGIT GAACGIGGAC AAGI</u>TCGCGI IGG.....IAG CINAA<u>CCAGG IACAAICACA CCICRIA</u>CAA

Enterococcus

faecalis faecium

- 55 -

	Haemophilus	AGGTCGTGTA	GAACGAGGTA	TTATCCGTAC	AGG	TAG.	CGAAACCAGG	AGGT <u>CGTGTA GAACGAGGT</u> A TT <u>ATC</u> CGTAC AGGTAG CGAAACCAGG TTCAATCAAA	200 40 40 400		
	influenzae						1	4	פרטערערער פרי	15/	
	Helicobacter	AGGTAGGATT	GAAAGAGGCG	TGGTGAAAGT	AGG	TAT	SCAPACCAGG	AGGIA <u>ggaII GAAAGagg</u> CG IGG <u>IGAAA</u> GI AGGIRT GCAAACCAGG WYCWAWAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA		į	
	pylori							TANK TO THE PARTY OF THE PARTY	CC CACA	158	
S	Listeria	TGGACGTGTT	GAACGIGGAC	AAGITAAAGT	TGG	TAG (	CTAAACCAGG	TGGA <u>CSTGTT GAACGIGGAC AAGTTAAA</u> GT TGGTAG CTAAACCAGG TTCGATTACT CCACACA	***************************************		
	monocytogenes			· .					HT JUNE TO SERVICE THE	138	
	Micrococcus	CGGTCGCGCC	GAGCGCGCCA	CCCIGAAGAT	CAA	TGG	NGGAGCCGGG	COGT <u>CG</u> CGC GAGCGCGCA CCCTGAAGAT CAATGG TGGAGCCGGG CTCCATCACC			
	luteus							770700750	ברפרשרשרנים	159	
	Mycobacterium	CGGACGTGTG	<u>GAGCGCGG</u> CG	TGATCAACGT	GAA	TCA.	CAAGCCCGG	CGGA <u>CGIGI</u> G GAG <u>CGCG</u> CG TGAICAACGT GAATCA CCAAGCCCGG CACCAACAA		Ş	
2	tuberculosis								500000000000000000000000000000000000000	160	
	Mycoplasma	AGGAAGAGII	GAAAGAGGIG	AACTCAAAGT	AGG	TAG C	AAAACCAGG	AGGAAGAGII GAAAGAGGIG AACICAAAGI AGGIAG CAAAACCAGG CICINITATAA	***************************************	;	
	genitalium							S SUCCESSION OF THE PERSON OF	POPPE POP	191	
	Neisseria	CGG <u>CCGTGIA GAGCGAGGI</u> A TC <u>AICCA</u> CGI IGGIGG CCAAACGGGG IACIANCACT CTTCAAACA	<u>GAGCGAGGTA</u>	TCATCCACGT	TGG	TGG C	CAAACGGGG 1	ACTATCACT .		(	
	gonorrhoeae							1		797	
'n	Salmonella	CGGT <u>CGTGTA GAGCGCGGT</u> A TC <u>AICAAA</u> GT GGG IGG CTAAGCCGGG CACCATGAAC	GAGCGCGGTA	TCATCAAAGT	366	TGG	TAAGCCGGG	7 6 6 7 6 7 7 6 7 7 6		į	
	typhimurium							A DAMESTON	Ferrence A	164	
	Shewanella	AGGI <u>CGIGIT GAGGGIGGI</u> A TIGIACGCGT AGGIAG CGAAGCCAGG TFCAAFFARA	SAGCGTGGTA	TGIACGCGT	JOG.	TAG	T SERVICE T	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0			
	putida						¥	T THE THEFT	CACACACTA	165	
	Staphylococcus	AGGCCSTGIT GAACGIGGIC AAAICAAAGI IGGIAG CIGCICCTGG TICAATTAAAGI IGG	SAACGTGGTC 1	MATCAAAGT		TAG	TGCTCCTGG T	C antipod C		:	
0	aureus							N WINTERSON		140	
	Staphylococcus	AGG <u>CCGIGIT GAACGIGGIC AAAICAAAG</u> T WGGTAG CTGCTTCTGG TTCTTAG	PAACGIGGIC 2	AATCAAAGT V	lgg.	F PAG	<u></u>				
	epidermidis					; !	-	ם שושוישוא		141	
	Staphylococcus	AGG <u>CCGIGIT GAACGIGGIC AAATCAAA</u> GT CGGIRG CIGCTCCTGG IACTARCACA CAACAAAGA	AACGIGGIC A	AATCAAAGT C	gg	TAG C	recrease ry	יט אטשמשפשטי		;	
	saprophyticus							3		142	
	Staphylococcus	AGG <u>CCGTGTT SAACGIGGIC AAAICAAA</u> GT CGGTAG CAGCTCCTGG CTCM2 TTA CAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	AACGTGGTC A	AATCAAAGT O	GG	TAG CA	m semonas	Compare and Compar			
,	simulans						2000000	בייניים בייניים		143	

- 56 -

BNSDOCID: <WO 9820157A2 1 >

SEQ ID NO: 18b SEQ ID NO: 17 genus-specific

10 Selected primer

TRIGIGGI GIRATWGWRC CAGGAGC CCGIGIT GAACGIGGIC AAAICAAA

- 57 -

15 The sequence numbering refers to the S.aureus tuf gene fragment. Underlined nucleotides are identical to the selected sequence or match that sequence.

"R", "W" and "Y" designate nucleotide positions which are degenerated. "R" stands for A ox G; •

This sequence is the reverse complement of the above tuf sequence. "W", for A or T; "Y", for C or T.

sequences\*:

Strategy for the selection from tuf sequences of the amplification primers specific for the species Candida albicans (continues on pages 59 and 60). Annex IV:

8 o	e.	8	m						
120	121	122	123	124		153	126	132	154
213 SEQ AGACCITGTT	AGAYCTTGTT	AGACCTTATT	AGACCTIGIT	AGACT <u>T</u> TGTT	AGACTOTTT	<u>ag</u> ctactgga <u>ag</u> ctgatgaa	CTCATGCA	ATTAATGGC	ACTGGCTGG
58 COTCAAGAAG GITGGITACA ACCCAAAGAC TGTCAA AICCGGITAAA GITACIGGIA AGACCITGTA	CAT <u>CAAGAAG GICGGITACA ACCCAAAGA</u> C TGICAA GG <u>CTGGIGGC GICAAGGGIA AGAYCI</u> TGIT	Chi <u>caagaag sitgsfiaca acceaaag</u> ac tstcaa ge <u>cagsf</u> sft <u>sitaaggsta agacet</u> natt	cot <u>caagaag strostrac</u> a <u>accetaa</u> ngc tottaa a <i>getssta</i> ag <i>stra</i> ecss <u>ea agace</u> tott	COTCAAGAAG GIIGGIIACA ACCCIAAGGC TOTCAA GGCTGGIAAG GIIACCGGIA AGACIITOTI	CATCAAGAAG GICGGIITCA ACCCCAAGAC CGICAA GGCIGGIGTC GICAAGGGIA AGACICTITT	GGAGAICCG GAGCTGCTCA CCAAGTTGG CTAGTT AGGCCTQAGA TCTGTGCAGA AGCTACTGGA GGAGCTGCGC GAGCTGCTCA GCAAGTACGG CTTCAA AIGAAGTACGG AGCTGATGAA	TCCATCATCG 2	AAAATCTTAG A	AAAATCCTGG A
1 A ATCCGGTAAA	A GG <u>CTGGT</u> GTC	. GG <u>CAGGI</u> GTT	AGCTGGTAAG	GGCTGGTAAG	GG <u>CT<u>GGT</u>GTC</u>	AGGCCTGAAG AIG	GTGGACCC <u>A</u> G	TGAAGAA	· · GGGAAGCG
181 CAA	C.		TAA	CAA	CAA	GTT	GAA	:	Ė
90 7 TGT.	TGT.	TGT.	TGT.	TGT.	CGT.	A F	E.	TTT.	CHT.
ACCCAAAGA	ACCCAAAGA	ACCCAAAGAC	ACCCTAAAGO	ACCCTAAGGC	ACCCCAAGAC	CCGAGTTTGG GCAAGTACGG	CTGAGC <u>AG</u> GA	caga <u>ata</u> cga	TCAGTACGA
GTIGGITACA	GICGGITACA	GITGGITACA	GIIGGIIACA	GIIGGIIACA	GICGGIITCA	GAGCTGCT <u>CA</u> GAGCTGCT <u>CA</u>	SAGCTGCTCG	PACTTA <u>T</u> TAT (	AACTECTGT C
58 CGT <u>Caagaag</u>	CATCAAGAAG	CAT <u>CAAGAAG</u>	CGTCAAGAAG	CGTCAAGAAG	CAT <u>CAAGAAG</u> e	GGAG <u>A</u> TCCG <u>G</u> GGAGCT <u>G</u> CGC	GGAGATCCRT GAGCTGCTCG CTGAGCAGGA TTAGAA GTGGACCCAG TCCATCATCG ACCTGATGCA	GGAAGTICOT GACTIALIAT CAGAALACGA TITTGAAGAA AAAATCIIAG BATTAATGGC	GGAAGTICGT GAACTICTGT CTCAGTACGA CTIGGGAAGCG AAAATCCTGG BACTGGCTGG
<u>Candida</u> al <u>bicans</u>	Candida 5 glabrata	Candida krusei	Candida parapsilosis	10 Candida tropicalis	Schizo- saccharomyces pombe	Human 15 Chlamydia trachomatis	Corynebacterium diphteriae	Enterococcus 20 faecalis	Escherichia coli

- 58 -

140 145 169 162 164 138 159 135 157 156 ggragitogi gactia<u>t</u>ira goğra<u>ta</u>iga cit..... ...<u>og</u>rag<u>ra</u> arabicitag battariggr ggaa<u>a</u>tccgt <u>G</u>accta<u>t</u>tgt caga<u>atb</u>cga ctt..... ...<u>cg</u>aag<u>a</u>c a<u>r</u>cgtatgg <u>a</u>attgatgaa agagegege <u>gaig</u>egette cigg<u>ala</u>igs gii...gga ggai<u>g</u>eagci ig<u>iatig</u>agg <u>a</u>acigetige ggragiccsi grstisciss cisccsassa ait...cra sissgeicsas icisicacac agiisaissa GGAAAICCGC GACTGCIGI CCAGCIACGA CII..... ..A<u>CGAAGAA</u> AAAAICTICG AACIGGCIAC GGAAGIICGC GAACIGCIGI CICAGIACGA CII..... ..GGGAAGCG AAAAICAICG AACIGGCIGG ggaagttogt ga<u>t</u>ctaita<u>a</u> ctgaatatga att........gggaagct aaa<u>attg</u>acg <u>ag</u>ttaatgga CGAGGITCGC GAAGAACTGA CTAAACGCGG ITT...... ..GGGI<u>TAAA</u> GAAAT<u>IG</u>AAA ACCTGANGGA agagetoget gacctoctog <u>aagaaaa</u>ggg cit...caa g<u>e</u>gggtag<u>a</u>g accetoaagg <u>aacto</u>atgaa ggragticot gaact<u>i</u>ctat ct<u>caata</u>tga ctt..... ..gggaag<u>aa</u> aaa<u>a</u>tcct<u>t</u>g <u>ag</u>ttagcaaa 15 Staphylococcus Flavobacterium Streptococcus monocytogenes gonorrhoeae typhimurium Micrococcus ferrugineum Gardnerella 5 Haemophilus pneumoniae Salmonella influenzae Neisseria Treponema vaginalis 20 pallidum Listeria aureus 10 luteus

59 -

- 60 -

ATCCGGTAAA GTTACTGGTA AGACCT	SBQ ID NO: 12*	AGGICTIACC AGTAACTITAC CGGAI		10 The sequence numbering refers to the Candida albicans tuf gene fragment. Underlined nucleotides are
CAAGAAG GTTGGTTACA ACCCAAAGA	SEQ ID NO: 11	CAAGAAG GTTGGTTACA ACCCAAAGA	mar o p	ing refers to the Candida albicans tu
. Selected sequences	Selected 5 species-specific	primer sequences:	!	10 The sequence number:

identical to the selected sequence or match that sequence.

This sequence is the reverse-complement of the above tuf sequence.

TTAGAGAIIG COGAIGCCII AGIIICAAGI GGTGC...AGCTC GACIAAIGTC ICAAGGACTA CGIAAATTAT CTOGAAAICT GTGAQGCCCI GGCGCGTTCT GGCGC...GGCRC GTAIGAIGAG CCAGGGGAIG CGTAAGCTGG

agglomerans Enterococcus

20 faecium Escherichia coli

Strategy for the selection from the reck gene of the amplification primers specific for the genus Straptococcus (continues on pages 62 and 63).	415 449540 574 SEQ 1D NO	CTCEAGAICA CVGAAGGCGCT GGIGCGCTCG GGCTCGGCCC GCC <u>TGAAGAAG CCAAGGGGTIG GGCAA</u> GCTGA	CHICARAMICA COGRAGOGOT GARBOCOTIC GOUTCGOCCC GUCTGARGTC GEAGGGOTG CGUAAGTAA	TTR <u>GRARING</u> T <u>rgrarict</u> acceans accocaccea gro <u>ttrigi</u> ct <u>Icragc</u> tc <u>ia agrar</u> etta	TTGAGT <u>AING CAGAGCTCII</u> AGCGCGTTCT GGAGCAGCTC GC <u>AIGANG</u> TT G <u>CAGGCTCIA <u>C</u>GCAAATTAA</u>	S TTA <u>GRABINA CAGRA</u> GCTII AGIINGATCA GGAGCAGCTA GAI <u>INAIG</u> TC A <u>CARGCCTIA AGRAB</u> GTIAA	s erium cro <u>gadate cabala</u> toc <u>t tott</u> coctct geagcagcsc ott <u>roatsag tcags</u> sc <u>tg cetaa</u> galea	rrulosis car crogadart gegalgeget gaccostor gegecagete grangargag c <u>eaggearg cgraag</u> ettg
Annex V: Strateg	41	5 · Bordetella CT	pertussis Burkholderia CT	cepacia Campylobacter T	10 jejuni Chlamydia T	trachomatis Clostridium T	perfringens 15 Corynebacterium C	pseudotuberculosis Enterobacter C
~		ú			10		15	

- 61 -

- 62 -

34	35	36			
CTTEMENTIC COGGRADANT GATTEMETEN GOTGEGGCTC GTATGATGAG CCAGGCCANG CGTADACTTG	CTIGAAITG CAGGTAAAIT GAITGAITCT GGTGCAGCAC GTAIGAIGAG ICAGGCGAIG CGTAAATTAT 35	CTCGAAAITG CAGGITAACK SAXIGACTCT GGTGCAGCGC GTAIGAAGAG ICAAGCGAIG GGIAAACTTT 36	CGTAAACTGA	CGTAAGCTGG	
CCAGGCCATG	TCAGGCCATG	TCAAGCCATG	GCAAGCAATG	CCAGGCTATG	
GTATGATGAG	GTATGATGAG	GTATGATGAG	CTG <u>GAAAI</u> TT GTGAIGCACI GGGICGCTCT GGTGCAGCGC GT <u>AIGTIG</u> TC G <u>CLAAGCAAIG CGTAA</u> ACTGA	CTGGAAAIIT GTGAIGGGGT GAAIGGTGT GGTGCCGCGC GT <u>AIGAIGAG</u> <u>CAAGGGTAIG CGIAA</u> GCTGG	
GGCTC	AGCAC	AGCGC	AGCGC	09090	
GGTGC.	GGTGC.	GGTGC.	GGTGC.	GGTGC.	
GATTGACTCA	GATTGATTCT	GATTGACTCT	gec <u>r</u> cecrer	<u>Gact</u> cgctct	
CGGGAAAATI	CAGGTAAATT	CAGGTAAGCT	GTGAIGCACI	GTGATGCGCT	
CTTGAGATTG	CTTGAAATTG	CTCGBAATIG	CTGGAAALTT	CTGGAAATTT	
Streptococcus	pneumoniae Streptococcus	pyogenes Streptococcus	<u>salivarius</u> Vibrio	cholerae Yersinia	10 pestis
		Ŋ			10

	Selected	SEQ ID NO: 21	SEQ ID NO:
15	15 genus-specific		
	primer	GAAATTG CAGGIAAATT GATTGA	TTACGCAT GGCITGACT
	sednences,:		

22b C ATCAT

63 -

ATGATGAG TCAIGCCATG CGTAA

GARATTG CAGGIARATT GATTGA

Selected sequences\* The sequence numbering refers to the S.pneumoniae recA sequence. Underlined nucleotides are identical 20 to the selected sequence or match that sequence.

- "I" stands for inosine which is a nucleotide analog that can bind to any of the four nucleotides G or T. A, C,
  - This sequence is the reverse complement of the above rech sequence.

25

Annex VI: Specific and ubiquitous primers for DNA amplification

- 64 -

				LICACION
	SEQ	ID NO Nucleotide sequence	Originating	DNA fragment
			SEQ ID	Nucleotide
			NO	position
5	Bact	erial species: Enterococcus faecium		Postcion
5	_			
	1 2 <sup>b</sup>	5'-TGC TTT AGC AAC AGC CTA TCA G	26*	273-294
	2"	5'-TAA ACT TCT TCC GGC ACT TCG	26ª	468-488
	Bacte	erial species: Listeria monocytogenes		
10		Disteria monocytogenes		
	3	5'-TGC GGC TAT AAA TGA AGA GGC		
	4 <sup>b</sup>	5'-ATC CGA TGA TGC TAT GGC TTT	27*	339-359
			27*	448-468
45	Bacte	erial species: Neisseria meningitidis		
15				
	5	5'-CCA GCG GTA TTG TTT GGT GGT	284	56-76
	6ь	5'-CAG GCG GCC TTT AAT AAT TTC	28*	212-232
	Bacte	rial monice at the		
20	Duces	rial species: Staphylococcus saprophyt:	icus	
	7	5' - AGA TCG AAT TCC ACA TGA AGG TTA T		
	8p	5' - TCG CTT CTC CCT CAA CAA TCA AAC T	TA TGA 29°	290-319
		TO CAR ICA AAC TA	AT CCT 29°	409-438
22.00	Bacter	rial species: Streptococcus agalactiae		
25			*	
	9	5'-TTT CAC CAG CTG TAT TAG AAG TA	30ª	59-81
	10 <sup>b</sup>	5'-GTT CCC TGA ACA TTA TCT TTG AT	30*	190-212
	Euros I	l amazini a a a a		
30	E MILEGIA	species: Candida albicans		
	11	5'-CAA GAA GGT TGG TTA CAA CCC AAA GA		
	12 <sup>b</sup>	5'-AGG TCT TAC CAG TAA CTT TAC CGG AT	120°	61-86
•		THE CAS TAR CTT TAC CGG AT	120°	184-209

Sequences from databases.

<sup>35</sup> b These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.

c Sequences determined by our group.

Annex VI: Specific and ubiquitous primers for DNA amplification (continues on next page)

SEQ II	NO Nucle	otide se	quence			Originating	DNA fragment
						SEQ ID	Nucleotide
						NO	position
Bacte	cial genus:	Enteroco	ccus				
13	5'-TAC TGA	CAA ACC	ATT CA	T GAT	G	131-134 <sup>a,b</sup>	319-340°
14ª	5'-AAC TTC	GTC ACC	AAC GC	G AAC		131-134ª,b	410-430°
Bacte	rial genus:	Neisseri	a				
15	5'-CTG GCG	CGG TAT	GGT CG	G TT		31°	21-40 <sup>f</sup>
16 <sup>d</sup>	5'-GCC GAC				G	31°	102-123f
	rial genus:						
17	5'-CCG TGT	TGA ACG	TGG TG	TAA AC	CAA A	140-143 <sup>a,b</sup>	391-415 <sup>9</sup>
184	5'-TRT GTO						584-608 <sup>9</sup>
19	5'-ACA ACC						562-583 <sup>g</sup>
204	5'-ACC AT						729-753 <sup>g</sup>
Bacte	rial genus:	Strepto	coccus				
- 21	5'-GAA AT	r gca ggi	AAA T	TG ATT	GA	32-36°	418-440h
22 <sup>d</sup>	5'-TTA CG	C ATG GCI	TGA C	TC ATC	AT	32-36°	547-569h
•	•	Univers	al pri	mers			
23	5'-ACI KK	I ACI GG	GTI G	AR ARG	TT	118-146 <sup>a,b</sup>	493-5151
244	5'-AYR TT	יד ידריד כירי	r GGC A	TI ACC	AT	147-171 <sup>a,e</sup> 118-146 <sup>a,b</sup>	778-800°
24-	5 -AIR II	1 101 00.				147-171ª.ª	

- 30 \* These sequences were aligned to derive the corresponding primer.
  - b tuf sequences determined by our group.
  - $^{\circ}$  The nucleotide positions refer to the E. faecalis tuf gene fragment (SEQ ID NO: 132).
- These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.
  - Sequences from databases.
  - $^{\rm t}$  The nucleotide positions refer to the N. meningitidis asd gene fragment (SEQ ID NO: 31).

- $^{\rm g}$  The nucleotide positions refer to the S. aureus tuf gene fragment (SEQ ID NO: 140).  $^{\circ}$
- $^{\rm h}$  The nucleotide positions refer to the S. pneumoniae recA gene (SEQ ID NO: 34).
- 5  $^{\rm i}$  The nucleotide positions refer to the E. coli tuf gene fragment (SEQ ID NO: 154).

Annex VI: Specific and ubiquitous primers for DNA amplification

SEQ ID	NO Nucleotide sequence	Originating	DNA fragment
		SEQ ID	Nucleotide
		NO	position
Antibi	otic resistance gene: blatem		
37	5'-CTA TGT GGC GCG GTA TTA TC	-	-
38	5'-CGC AGT GTT ATC ACT CAT GG	-	-
39	5'-CTG AAT GAA GCC ATA CCA AA	-	-
40	5'-ATC AGC AAT AAA CCA GCC AG	-	-
Antibi	otic resistance gene: blashv		
41	5'-TTA CCA TGA GCG ATA ACA GC	-	-
42	5'-CTC ATT CAG TTC CGT TTC CC	-	-
43	5'-CAG CTG CTG CAG TGG ATG GT	-	-
44	5'-CGC TCT GCT TTG TTA TTC GG	-	-
Antib	iotic resistance gene: blarob		
45	5'-TAC GCC AAC ATC GTG GAA AG	-	-
46	5'-TTG AAT TTG GCT TCT TCG GT	-	-
47	5'-GGG ATA CAG AAA CGG GAC AT	-	-
48	5'-TAA ATC TTT TTC AGG CAG CG	-	-
Antib	iotic resistance gene: blaoxa		
	5'-GAT GGT TTG AAG GGT TTA TTA TAA G	110°	686-71
49	5'-GAT GGT TTG AAG GGT TTA TTA	110ª	802-82
50 <sup>b</sup>			
Antil	piotic resistance gene: blaz		
51	5'-ACT TCA ACA CCT GCT GCT TTC	1114	511-53
52b	mga gga agg	111*	663-68
Anti	biotic resistance gene: aads		
53	5'-GGC AAT AGT TGA AAT GCT CG	-	-
54	5'-CAG CTG TTA CAA CGG ACT GG	-	-
Anti	biotic resistance gene: aacC1		
55	5'-TCT ATG ATC TCG CAG TCT CC	-	-
56	5'-ATC GTC ACC GTA ATC TGC TT		

Sequences from databases.

These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex VI:	Specific and ubiquitous primer	s for DNA ampli	fication
SEQ ID NO	Nucleotide sequence	Originating	DNA fragmen
			Nucleotide
2		NO	position

	PA ID NO MUCIEOLIGE seduence	Originating DNA fragment			
		SEQ ID	Nucleotide		
- A1	ntibiotic resistance gene: aacC2	NO	position		
5	aacC2				
	57 5'-CAT TCT CGA TTG CTT TGC TA				
	58 5'-CCG AAA TGC TTC TCA AGA TA	-	-		
	TOTAL TOTAL TEN AGA TA	-	-		
Ar	ntibiotic resistance gene: aacC3				
	aaccs				
5	9 5'-CTG GAT TAT GGC TAC GGA GT				
6	0 5'-AGC AGT GTG ATG GTA TCC AG	-	-		
	The second	-	-		
An	tibiotic resistance gene: aac6'-IIa				
-	1 5'-GAC TCT TGA TGA AGT GCT GG	112ª			
6	2b 5'-CTG GTC TAT TCC TCG CAC TC	112*	123-142		
			284-303		
6	- III GIZ IIIG GCA GGA TTC GT	112*	445 44.		
6	4b 5'-GCT TTC TCT CGA AGG CTT GT	112*	445-464		
			522-541		
An	tibiotic resistance gene: aacA4				
6	5'-GAG TTG CTG TTC AAT GAT CC				
66	5'-GTG TTT GAA CCA TGT ACA CG	-	-		
	The sea were	-	-		
Ant	ibiotic resistance gene: aad(6')				
114.5	make a management of the same and the same a				
17	3 5'-TCT TTA GCA GAA CAG GAT GAA	i i ni i ni i ni			
17	4 5'-GAA TAA TTC ATA TCC TCC G	_	-		
			-		
	ibiotic resistance gene: vanA				
67	TOT NOW GGT CIA GCC CGT GT	_			
68	5'-ACG GGG ATA ACG ACT GTA TG	-	_		
			=		
	5'-ATA AAG ATG ATA GGC CGG TG	_	_		
70	5'-TGC TGT CAT ATT GTC TTG CC	-	-		
	***				
Ant	ibiotic resistance gene: van8				
71	5'-ATT ATC TTC GGC GGT TGC TC	116*	22-41		
721	5'-GAC TAT CGG CTT CCC ATT CC	116ª	171-190		
	F1 #F5				
73 74¹	THE PART CAG GAC AA	116ª	575-594		
74.	5'-CTG ATG GAT GCG GAA GAT AC	116	713-732		

a Sequences from databases.

## SUBSTITUTE SHEET (RULE 26)

 $<sup>^{\</sup>mathtt{b}}$  These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex VI: Specific and ubiquitous primers for DNA amplification

SEO	ID NO Nucleotide sequence	Originatin	g DNA fragment
		SEQ ID	Nucleotide
		NO	position
Anti	biotic resistance gene: vanC		
	5'-GCC TTA TGT ATG AAC AAA TGG	117ª	373-393
75 76b		117*	541-563
76-	5 - GIG ACT 1111 CTC 1120 CCC 1120		
Anti	biotic resistance gene: msrA		
77	5'-TCC AAT CAT TGC ACA AAA TC	-	-
78	5'-AAT TCC CTC TAT TTG GTG GT	-	-
79	5'-TCC CAA GCC AGT AAA GCT AA	-	-
80	5'-TGG TTT TTC AAC TTC TTC CA	-	-
Ant	biotic resistance gene: satA		
81	5'-TCA TAG AAT GGA TGG CTC AA	-	-
82	5'-AGC TAC TAT TGC ACC ATC CC	-	-
Ant	ibiotic resistance gene: aac(6')-aph(2"	)	
	5'-CAA TAA GGG CAT ACC AAA AAT C	_	_
83 i 84	The same was command of	-	-
84			
85		-	<del>-</del>
86	5'-CCT TTA CTC CAA TAA TTT GGC T	-	-
) Ant	ibiotic resistance gene: vat		
	5'-TTT CAT CTA TTC AGG ATG GG	_	_
87 88		-	-
	, 5 00.1 00.0 11		
5 Ant	ibiotic resistance gene: vga		
. 85	5'-TGT GCC TGA AGA AGG TAT TG	-	-
90	5'-CGT GTT ACT TCA CCA CCA CT	-	-
0 <u>An</u>	tibiotic resistance gene: ermA		
9	1 5'-TAT CTT ATC GTT GAG AAG GGA TT	113*	370-392
-	2b 5'-CTA CAC TTG GCT TAG GAT GAA A	113ª	487-508

<sup>45 \*</sup> Sequences from databases.

These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.

Annex VI: Specific and ubiquitous primers for DNA amplification

- 70 -

SEQ	ענ	NO	NGCT	eoti	de s	edne	nce				Origin:	ating DNA nt
											SEQ	Nucleotide position
Ant:	ibic	tic re	sist	ance	gen	<u>e</u> :	ermB					
93		5'-CTA	TCI	GAT	TGT	TGA	AGA	AGG	ATT		114*	266 222
94 <sup>b</sup>		5'-GT1									114	366-389 484-507
Anti	bio	tic re	sist	ance	gen	g: e.	rmC					
95		5'-CTT	GTT	GAT	CAC	GAT	AAT	TTC	С		115*	214 222
96b		5'-ATC	TTT	TAG	CAA	ACC	CGT	ATT	C		115*	214-235 382-403
Anti	bio	tic re	sist	ance	gene	2: 1	necA					
97		5'-AAC	AGG	TGA	ATT	ATT	AGC	ACT	TGT	AAG	_	
98		5'-ATT	GCT	GTT	AAT	ATT	TTT	TGA	GTT	GAA	-	-
Anti	bio	ic res	sista	nce	gene	2: 1	nt					
99		5'-GTG	ATC	GAA	ATC	CAG	ATC	С			_	_
100		5'-ATC	CTC	GGT	TTT	CTG	GAA	G			-	-
101		5'-CTG									-	_
102		5'-GAT	GTT	ACC	CGA	GAG	CTT	G			-	-
Antil	oiot	ic res	ista	nce	gene	: s	u1				 - 111	
103	9	-TTA	AGC	GTG	CAT	AAT	AAG	cc			_	_
104	5	'-TTG	CGA	TTA	CTT	CGC	CAA	CT			-	-
105	5	'-TTT	ACT	AAG	CTT	GCC	CCT	TC			_	_
106	5	'-AAA	AGG	CAG	CAA	TTA	TGA	GC				-

<sup>35 \*</sup> Sequences from databases.

These sequences are from the opposite DNA strand of the sequence of the originating fragment given in the Sequence Listing.

- 71 -

#### SEQUENCE LISTING

### (1) GENERAL INFORMATION:

- (i) APPLICANT:
  - (A) NAME: INFECTIO DIAGNOSTIC (I.D.I.) INC.
  - (B) STREET: 2050, BOULEVARD RENE LEVESQUE OUEST, 4E ETAGE
  - (C) CITY: STE-FOY
  - (D) STATE: QUEBEC
  - (E) COUNTRY: CANADA
  - (F) POSTAL CODE (ZIP): G1V 2K8
  - (G) TELEPHONE: (418) 681-4343
  - (H) TELEFAX: (418) 681-5254
  - (A) NAME: BERGERON, MICHEL G.
  - (B) STREET: 2069 RUE BRULARD
  - (C) CITY: SILLERY
  - (D) STATE: QUEBEC
  - (E) COUNTRY: CANADA
  - (F) POSTAL CODE (ZIP): GIT 1G2
  - (A) NAME: PICARD, FRANCOIS J.
  - (B) STREET: 1245, RUE DE LA SAPINIERE
  - (C) CITY: CAP-ROUGE
  - (D) STATE: QUEBEC
  - (E) COUNTRY: CANADA
  - (F) POSTAL CODE (ZIP): G1Y 1A1
  - (A) NAME: OUELLETTE, MARC
  - (B) STREET: 1035 DE PLOERMEL
  - (C) CITY: SILLERY
  - (D) STATE: QUEBEC
  - (E) COUNTRY: CANADA
  - (F) POSTAL CODE (ZIP): G1S 3S1
  - (A) NAME: ROY, PAUL H.
  - (B) STREET: 28, RUE CHARLES GARNIER
  - (C) CITY: LORETTEVILLE
  - (D) STATE: QUEBEC
  - (E) COUNTRY: CANADA
  - (F) POSTAL CODE (ZIP): G2A 3S1
- (ii) TITLE OF INVENTION: SPECIES-SPECIFIC, GENIUS-SPECIFIC AND UNIVERSAL DNA PROBES AND AMPLIFICATION PRIMERS TO RAPIDLY DETECT AND IDENTIFY COMMON BACTERIAL AND FUNGAL PATHOGENS AND ASSOCIATED ANTIBIOTIC RESISTANCE GENES ...
- (iii) NUMBER OF SEQUENCES: 174
- (iv) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
    (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)
- (vi) PRIOR APPLICATION DATA:

```
(A) APPLICATION NUMBER: US 08/743,637
```

(B) FILING DATE: 04-NOV-1996

# (2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Enterococcus faecium
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

#### TGCTTTAGCA ACAGCCTATC AG

ي ال الدياد والمسائد المساعدة الما المعادد

22

- (2) INFORMATION FOR SEQ ID NO: 2:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 21 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Enterococcus faecium
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

### TAAACTTCTT CCGGCACTTC G

21

# (2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Listeria monocytogenes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

#### TGCGGCTATA AATGAAGAGG C

. 21

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)	
<ul><li>(vi) ORIGINAL SOURCE:</li><li>(A) ORGANISM: Listeria monocytogenes</li></ul>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:	
ATCCGATGAT GCTATGGCTT T	21
(2) INFORMATION FOR SEQ ID NO: 5:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDEDMESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
<ul><li>(vi) ORIGINAL SOURCE:</li><li>(A) ORGANISM: Neisseria meningitidis</li></ul>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:	
CCAGCGGTAT TGTTTGGTGG T	21
(2) INFORMATION FOR SEQ ID NO: 6:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 21 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
<ul><li>(vi) ORIGINAL SOURCE:</li><li>(A) ORGANISM: Neisseria meningitidis</li></ul>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:	
CAGGCGGCCT TTAATAATTT C	21
(2) INFORMATION FOR SEQ ID NO: 7:	
(i) SEQUENCE CHARACTERISTICS:	

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- 74 -

```
(ii) MOLECULE TYPE: DNA (genomic)
                  (vi) ORIGINAL SOURCE:
                                    (A) ORGANISM: Staphylococcus saprophyticus
                  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
     AGATCGAATT CCACATGAAG GTTATTATGA
                                                                                                                                                                                                                  30
      (2) INFORMATION FOR SEQ ID NO: 8:
                    (i) SEQUENCE CHARACTERISTICS:
                                  (A) LENGTH: 30 base pairs
                                  (B) TYPE: nucleic acid
                                  (C) STRANDEDNESS: single
                                  (D) TOPOLOGY: linear
                (ii) MOLECULE TYPE: DNA (genomic)
                (vi) ORIGINAL SOURCE:
                                 (A) ORGANISM: Staphylococcus saprophyticus
               (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
  TCGCTTCTCC CTCAACAATC AAACTATCCT
                                                                                                                                                                                                               30
  (2) INFORMATION FOR SEQ ID NO: 9:
                 (i) SEQUENCE CHARACTERISTICS:
                               (A) LENGTH: 23 base pairs
                               (B) TYPE: nucleic acid
                               (C) STRANDEDNESS: single
                              (D) TOPOLOGY: linear
                                                                                                    the result of the same and the same of the
             (ii) MOLECULE TYPE: DNA (genomic)
             (vi) ORIGINAL SOURCE:
                              (A) ORGANISM: Streptococcus agalactiae
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
TTTCACCAGC TGTATTAGAA GTA
                                                                                                                                                                                                            23
(2) INFORMATION FOR SEQ ID NO: 10:
               (i) SEQUENCE CHARACTERISTICS:
                             (A) LENGTH: 23 base pairs
                             (B) TYPE: nucleic acid
                            (C) STRANDEDNESS: single
                            (D) TOPOLOGY: linear
          (ii) MOLECULE TYPE: DNA (genomic)
          (vi) ORIGINAL SOURCE:
```

(A) ORGANISM: Streptococcus agalactiae

- 75 -	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:	
GTTCCCTGAA CATTATCTTT GAT	23
(2) INFORMATION FOR SEQ ID NO: 11:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 26 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE:  (A) ORGANISM: Candida albicans	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:	
CAAGAAGGTT GGTTACAACC CAAAGA	26
(2) INFORMATION FOR SEQ ID NO: 12:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 26 base pairs  (B) TYPE: nucleic acid  (C) STRANDENNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE: (A) ORGANISM: Candida albicans	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:	
AGGTCTTACC AGTAACTTTA CCGGAT	26
(2) INFORMATION FOR SEQ ID NO: 13:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: (c) cacid  (C) STR:ANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:	
TACTGACAAA CCATTCATGA TG	22
(2) INFORMATION FOR SEQ ID NO: 14:	

(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 21 base pairs

25

- 76 -

(B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:	
AACTTCGTCA CCAACGCGAA C	
(2) INFORMATION FOR SEQ ID NO: 15:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:	
CTGGCGCGGT ATGGTCGGTT 20	
(2) INFORMATION FOR SEQ ID NO: 16:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:	
GCCGACGTTG GAAGTGGTAA AG	
(2) INFORMATION FOR SEQ ID NO: 17:	
(i) SEQUENCE CHARACTERISTICS: (A) LEMOTH: 25 base pairs (B) TYPE: nucleic acid (C) STRANDEDMESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:	

SUBSTITUTE SHEET (RULE 26)

CCGTGTTGAA CGTGGTCAAA TCAAA

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

- 77 -

(B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:	
TRIGIGGIGI RAIWGWRCCA GGAGC	25
(2) INFORMATION FOR SEQ ID NO: 19:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 25 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDMESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:	
ACAACGTGGW CAAGTWTTAG CWGCT	25
(2) INFORMATION FOR SEQ ID NO: 20:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 25 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:	
ACCATTTCWG TACCTTCTGG TAAGT	25
(2) INFORMATION FOR SEQ ID NO: 21:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

EATURE:

(A) NAME/KEY: misc\_feature

(B) LOCATION:12 (D) OTHER INFORMATION:/note= "n = inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GAAATTGCAG GNAAATTGAT TGA

23

23

- 78 -

- (2) INFORMATION FOR SEQ ID NO: 22:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 23 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:12
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

### TTACGCATGG CNTGACTCAT CAT

- (2) INFORMATION FOR SEQ ID NO: 23:
- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:3
    - (D) OTHER INFORMATION:/note= "n.= inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
      (B) LOCATION:6
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:9
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:12
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION: 15
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

# ACNKKNACNG GNGTNGARAT GTT

- (2) INFORMATION FOR SEQ ID NO: 24:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 23 base pairs
      - (B) TYPE: nucleic acid
      - (C) STRANDEDNESS: single
      - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:6
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION: 9
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:12
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION: 18
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

AYRTTNTCNC CNGGCATNAC CAT

- (2) INFORMATION FOR SEQ ID NO: 25:

  - (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 10 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

TCGCTTCTCC

(2) INFORMATION FOR SEQ ID NO: 26:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 600 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double

23

. . . .

23

10

ı	D.	)	TO	PO	T.O	av.	. 1	in	

(ii)	MOLECULE	TYPE:	DNA	(genomic

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Enterococcus faecium

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

TTCTTAGAGA CATTGAATAT GCCTTATGTC GGCGCAGGCG TATTGACCAG TGCATGTGCC	6
ATGGATAAAA TCATGACCAA GTATATTTTA CAAGCTGCTG GTGTGCCGCA AGTTCCTTAT	12
GTACCAGTAC TTAAGAATCA ATGGAAAGAA AATCCTAAAA AAGTATTTGA TCAATGTGAA	18
GGTTCTTTGC TTTATCCGAT GTTTGTCAAA CCTGCGAATA TGGGTTCTAG TGTCGGCATT	24
ACAAAGGCAG AAAACCGAGA AGAGCTGCAA AATGCTTTAG CAACAGCCTA TCAGTATGAT	300
TCTCGAGCAA TCGTTGAACA AGGAATTGAA GCGCGCGAAA TCGAAGTTGC TGTATTAGGA	360
AATGAAGATG TTCGGACGAC TTTGCCTGGC GAAGTCGTAA AAGACGTAGC ATTCTATGAT	420
TATGAAGCCA AATATATCAA TAATAAAATC GAAATGCAGA TTCCAGCCGA AGTGCCGGAA	480
GAAGTTTATC AAAAAGCGCA AGAGTACGCG AAGTTAGCTT ACACGATGTT AGGTGGAAGC	540
GGATTGAGCC GGTGCGATTT CTTTTTGACA AATAAAAATG AATTATTCCT GAATGAATTA	600
(2) INFORMATION FOR SEQ ID NO: 27:	

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1920 base pairs the control of the cont
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Listeria monocytogenes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GTGGGATTAA ACAGATTTAT GCGTGCGATG ATGGTGGTTT TCATTACTGC CAATTGCATT 60 ACGATTAACC CCGACATAAT ATTTGCAGCG ACAGATAGCG AAGATTCTAG TCTAAACACA 120 GATGAATGGG AAGAAGAAA AACAGAAGAG CAACCAAGCG AGGTAAATAC GGGACCAAGA 180 TACGAAACTG CACGTGAAGT AAGTTCACGT GATATTAAAG AACTAGAAAA ATCGAATAAA 240 GTGAGAAATA CGAACAAAGC AGACCTAATA GCAATGTTGA AAGAAAAAGC AGAAAAAGGT 300 CCAAATATCA ATAATAACAA CAGTGAACAA ACTGAGAATG CGGCTATAAA TGAAGAGGCT 360

TCAGGAGCCG ACCGACCAGC TATACAAGTG GAGCGTCGTC ATCCAGGATT GCCATCGGAT	420
AGCGCAGCGG AAATTAAAAA AAGAAGGAAA GCCATAGCAT CATCGGATAG TGAGCTTGAA	480
AGCCTTACTT ATCCGGATAA ACCAACAAAA GTAAATAAGA AAAAAGTGGC GAAAGAGTCA	540
GTTGCGGATG CTTCTGAAAG TGACTTAGAT TCTAGCATGC AGTCAGCAGA TGAGTCTTCA	600
CCACAACCTT TAAAAGCAAA CCAACAACCA TTTTTCCCTA AAGTATTTAA AAAAATAAAA	660
GATGCGGGA ANTGGTACG TGATAAAATC GACGAAAATC CTGAAGTAAA GAAAGCGATT	720
GTTGATAAAA GTGCAGGGTT AATTGACCAA TTATTAACCA AAAAGAAAAG	780
AATGCTTCGG ACTTCCCGCC ACCACCTACG GATGAAGAGT TAAGACTTGC TTTGCCAGAG	840
ACACCAATGC TTCTTGGTTT TAATGCTCCT GCTACATCAG AACCGAGCTC ATTCGAATTT	900
CCACCACCAC CTACGGATGA AGAGTTAAGA CTTGCTTTGC CAGAGACGCC AATGCTTCTT	960
GGTTTTAATG CTCCTGCTAC ATCGGAACCG AGCTCGTTCG AATTTCCACC GCCTCCAACA	1020
GAAGATGAAC TAGAAATCAT CCGGGAAACA GCATCCTCGC TAGATTCTAG TTTTACAAGA	1080
GGGGATTTAG CTAGTTTGAG AAATGCTATT AATCGCCATA GTCAAAATTT CTCTGATTTC	1140
CCACCAATCC CAACAGAAGA AGAGTTGAAC GGGAGAGGCG GTAGACCAAC ATCTGAAGAA	1200
TTTAGTTCGC TGAATAGTGG TGATTTTACA GATGACGAAA ACAGCGAGAC AACAGAAGAA	1260
GAAATTGATC GCCTAGCTGA TTTAAGAGAT AGAGGAACAG GAAAACACTC AAGAAATGCG	1320
GGTTTTTTAC CATTAAATCC GTTTGCTAGC AGCCCGGTTC CTTCGTTAAG TCCAAAGGTA	1380
TCGAAAATAA GCGACCGGGC TCTGATAAGT GACATAACTA AAAAAACGCC ATTTAAGAAT	1440
CCATCACAGC CATTAAATGT GTTTAATAAA AAAACTACAA CGAAAACAGT GACTAAAAAA	1500
CCAACCCCTG TAAAGACCGC ACCAAAGCTA GCAGAACTTC CTGCCACAAA ACCACAAGAA	1560
ACCGTACTTA GGGAAAATAA AACACCCTTT ATAGAAAAAC AAGCAGAAAC AAACAAGCAG	1620
TCAATTAATA TGCCGAGCCT ACCAGTAATC CAAAAAGAAG CTACAGAGAG CGATAAAGAG	1680
GARATGARAC CACARACCGA GGARARARTG GTAGAGGARA GCGARTCAGC TARTARCGCA	1740
AACGGAAAAA ATCGTTCTGC TGGCATTGAA GAAGGAAAAC TAATTGCTAA AAGTGCAGAA	1800
GACGAAAAAG CGAAGGAAGA ACCAGGGAAC CATACGACGT TAATTCTTGC AATGTTAGCT	1860
ATTGGCGTGT TCTCTTTAGG GGCGTTTATC AAAATTATTC AATTAAGAAA AAATAATTAA	1920

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 415 base pairs

(B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
<ul><li>(vi) ORIGINAL SOURCE:</li><li>(A) ORGANISM: Neisseria meningitidis</li></ul>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:	
TACCGGTACG CTAAATATTG GTGATGTATT GGATATTATG ATTTGGGAAG CGCCGCCAGC	60
GGTATTGTTT GGTGGTGGCC TTTCTTCGAT GGGCTCGGGT AGTGCGCAAC AAACCAAGTT	120
GCCGGAGCAA CTGGTGACGG CACGTGGTAC GGTTTCTGTG CCGTTTGTTG GCGATATTTC	180
GGTGGTCGGT AAAACGCCTG GTCAGGTTCA GGAAATTATT AAAGGCCGCC TGAAAAAAAT	240
GGCCAATCAG CCGCAAGTGA TGGTGCGCTT GGTGCAGAAT AATGCGGCAA ATGTATCGGT	300
GATTCGCGCA GCCAATAGTG TGCGTATGCC GTTGACGGCA GCCGGTGAGC GTGTGTTGGA	360
TGCGGTGGCT GCGGTAGGTG GTTCAACGGC AAATGTGCAG GATACGAATG TGCAG (2) INFORMATION FOR SEQ ID NO: 29:	415
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 438 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
<ul><li>(vi) ORIGINAL SOURCE:</li><li>(A) ORGANISM: Staphylococcus saprophyticus</li></ul>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:	
TCGCTTCTCC AGAAGAAATT TTAGAAACAT ATCTAGAAAA TCCCAAATTA GATAAACCGT	60
TTATAMBANG MANAGEMENT OF THE STATE OF THE ST	00

TCGCTTCTCC AGAAGAAATT TTAGAAACAT ATCTAGAAAA TCCCAAATTA GATAAACCGT 60
TTATATTATG TGAATACGCA CATGCAATGG GAAATTCACC AGGAGATCTT AATGCATATC 120
AAACATTAAT TGAAAAATAT GATAGTTTTA TTGGCGGTTT TGTTTGGGAA TGGTGTGATC 180
ATAGCATTCA GGTTGGGATA AAGGAAGGTA AACCAATTTT TAGATATGGT GGAGATTTTG 240
GTGAGGCCTT ACATGACGGT AATTTTTGTG TTGATGGTAT TGTTTCGCCA GATCGAATTC 300
CACATGAAGG TTATATGAG TTTAAACATG AACATAGACC TTTGAGATTG GTTAACGAAG 360
AGGATTATCG GTTTACATTG AAGAATCAAT TTGATTTTAC AAATGCGGAG GATAGTTTGA 420
TTGTTGAGGG AGAAGCGA 438

(2) INFORMATION FO	SEO	ID	NO:	30
--------------------	-----	----	-----	----

# (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 768 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Streptococcus agalactiae
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

ATGAACGTTA	CACATATGAT	GTATCTATCT	GGAACTCTAG	TGGCTGGTGC	ATTGTTATTT	60
TCACCAGCTG	TATTAGAAGT	ACATGCTGAT	CAAGTGACAA	CTCCACAAGT	GGTAAATCAT	120
GTAAATAGTA	ATAATCAAGC	CCAGCAAATG	GCTCAAAAGC	TTGATCAAGA	TAGCATTCAG	180
TTGAGAAATA	TCAAAGATAA	TGTTCAGGGA	ACAGATTATG	AAAAACCGGT	TAATGAGGCT	240
					AGTTTATGAT	300
					AATCACTTTT	360
					ATTTGGGATA	420
					TAAAGCTCAA	480
	TABACCCATT	AGAACAAAAA	GTTTTAACTT	ATCCTGATTI	AAAACCAACT	540
					A TACACGCTTT	600
					AAATAAATT	660
					A ACAAGTTGAT	720
			r CAAACAGCA1			768

- (2) INFORMATION FOR SEQ ID NO: 31:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 421 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Neisseria meningitidis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ATGAAAGTAG GTTTCGTCGG CTGGCGCGGT ATGGTCGGTT CGGTTTTGAT GCAGCGTATG	60
AAAGAAGAAA ACGACTTCGC CCACATTCCC GAAGCGTTTT TCTTTACCAC TTCCAACGTC	120
GGCGGCGCAC GCCCTGATTT CGGTCAGGCG GCTAAAACAT TATTGGACGC GAACAACGTT	180
GCCGAGCTGG CAAAAATGGA CATCATCGTT ACCTGCCAAG GCGGCGACTA CACCAAATCC	240
GTCTTCCAAG CCCTGCGCGA CAGCGGCTGG AACGGCTACT GGATTGACGC GGCATCCTCG	300
CTGCGTATGA AAGACGACGC GATTATCGTC CTCGACCCCG TCAACCGCAA CGTCATCGAC	360
AACGGCCTCA AAAACGGCGT GAAAAACTAC ATCGGCGGCA ACTGTACCGT TTCCCTGATG	420
С	400
(2) INFORMATION FOR SEQ ID NO: 32:	421
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 213 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
<ul><li>(vi) ORIGINAL SOURCE:</li><li>(A) ORGANISM: Streptococcus gordonii</li></ul>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:	
TTCATAGACG CTGAGCACGC TTTGGATCCA TCTTACGCGG CTGCTCTAGG TGTAAATATT	60
GATGAGCTGT TGCTATCTCA ACCAGATTCT GGTGAGCAAG GTTTAGAAAT TGCAGGAAAA	120
TTGATTGACT CTGGGGCAGT TGATTTAGTT GTCATCGACT CTGTTGCAGC TCTTGTACCA	180
CGTGCGGAAA TCGATGGAGA TATCGGTGAT AGC	213
(2) INFORMATION FOR SEQ ID NO: 33:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 692 base pairs (B) TYPE: nucleic acid (C) STRANDEDMESS: double (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE: (A) ORGANISM: Streptococcus mutans	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:	
GGGCCGGAAT CTTCTGGTAA GACAACTGTC GCTCTTCATG CTGCTGCTCA GGCGCAAAAA	60

GATGGCGGTA	TTGCCGCTTT	CATTGATGCA	GAACATGCCC	TTGATCCAGC	CTATGCTGCT	120
	TTAATATTGA					180
					TGTTGACTCA	240
	TAGTACCACG					300
					CAATAAAACA	360
					GTTTGGTAAT	420
					TCTTGATGTC	480
					TGGTAAAGAG	540
					TTTTGTAGAA	600
					CAGTGATTTG	660
	AAAAAGCTGG					692
GGMMITATC						

- (2) INFORMATION FOR SEQ ID NO: 34:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 1204 base pairs
    - (B) TYPE: nucleic acid(C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Streptococcus pneumoniae
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:
- ATGGCGAAAA AACCAAAAAA ATTAGAAGAA ATTTCAAAAA AATTTGGGGC AGAACGTGAA 60 AAGGCCTTGA ATGACGCTCT TAAATTGATT GAGAAAGACT TTGGTAAAGG ATCAATCATG 120 CGTTTGGGTG AACGTGCGGA GCAAAAGGTG CAAGTGATGA GCTCAGGTTC TTTAGCTCTT 180 GACATTGCCC TTGGCTCAGG TGGTTATCCT AAGGGACGTA TCATCGAAAT CTATGGCCCA 240 GAGTCATCTG GTAAGACAAC GGTTGCCCTT CATGCAGTTG CACAAGCGCA AAAAGAAGGT 300 GGGATTGCTG CCTTTATCGA TGCGGAACAT GCCCTTGATC CAGCTTATGC TGCGGCCCTT 360 GGTGTCAATA TTGACGAATT GCTCTTGTCT CAACCAGACT CAGGAGAGCA AGGTCTTGAG 420 ATTGCGGGAA AATTGATTGA CTCAGGTGCA GTTGATCTTG TCGTAGTCGA CTCAGTTGCT 480 GCCCTTGTTC CTCGTGCGGA AATTGATGGA GATATCGGAG ATAGCCATGT TGGTTTGCAG 540 GCTCGTATGA TGAGCCAGGC CATGCGTAAA CTTGGCGCCT CTATCAATAA AACCAAAACA 600

ATTGCCATT	T TTATCAACC	ATTGCGTGA	AAAGTTGGAG	TGATGTTTG	AAATCCAGAA	
ACAACACCG	G GCGGACGTGC	TTTGAAATTC	TA MCCOMMON		TGTTCGTGGT	660
AATACACAA			AIGCITCAG	TCCGCTTGGA	TGTTCGTGGT	720
AMINCACAA	TTAAGGGAAC	TGGTGATCAA	AAAGAAACCA	ATGTCGGTAA	AGAAACTAAG	780
ATTAAGGTT	AATAAAAAT 3	GGTAGCTCCA	CCGTTTAAGG	AAGCCGTAGT	TGAAATTATG	840
TACGGAGAAC	GAATTTCTAA	GACTGGTGAG	CTTTTGAAGA	TTGCAAGCGA	TTTGGATATT	900
ATCAAAAAAG	CAGGGGCTTG	GTATTCTTAC	AAAGATGAAA	AAATTGGGCA	AGGTTCTGAG	960
AATGCTAAGA	AATACTTGGC	AGAGCACCCA	GAAATCTTTG	ATGAAATTGA	TAAGCAAGTC	1020
CGTTCTAAAT	TTGGCTTGAT	TGATGGAGAA	GAAGTTTCAG	AACAACAma -		1020
AAAGATGAGC	CAAAGAAAGA	ACARGO CO-		MACAAGATAC	TGAAAACAAA	1080
	CAAAGAAAGA	MGMAGCAGTG	AATGAAGAAG	TTCCGCTTGA	CTTAGGCGAT	1140
GAACTTGAAA	TCGAAATTGA	AGAATAAGCT	GTTAAAGCAG	TGGAGAAATC	CGCTACTTTT	1200
rcga						
(2) THEODIS	<b></b>					1204

- (2) INFORMATION FOR SEQ ID NO: 35:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 981 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:

  (A) ORGANISM: Streptococcus pyogenes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

ATGCGTTCAG GAAGTCTAGC TCTTGATATT GCTTGGATAG CTGGTGGTTA TCCTAAAGGA 60 CGTATCATCG AAATCTATGG TCCAGAGTCT TCCGGTAAAA CGACTGTGGC TTTACATGCT 120 GTAGCACAAG CTCAAAAAGA AGGTGGAATC GCAGCCTTTA TCGATGCCGA GCATGCGCTT 180 GATCCAGCTT ATGCTGCTGC GCTTGGGGTT AATATTGATG AACTTCTCTT GTCTCAACCA 240 GATTCTGGAG AACAAGGACT TGAAATTGCA GGTAAATTGA TTGATTCTGG TGCGGTTGAC 300 CTGGTTGTTG TCGATTCAGT AGCAGCTTTA GTGCCACGTG CTGAAATTGA TGGTGATATT 360 GGCGATAGCC ATGTCGGATT GCAAGCACGT ATGATGAGTC AGGCCATGCG TAAATTATCA 420 GCTTCTATTA ATAAAACAAA AACTATCGCA ATCTTTATCA ACCAATTGCG TGAAAAAGTT 480 GGTGTGATGT TTGGAAATCC TGAAACAACA CCAGGTGGTC GAGCTTTGAA ATTCTATGCT 540 TCTGTTCGGC TGGATGTGCG TGGAAACAAC CAAATTAAAG GAACTGGTGA CCAAAAGATA 600

GCCAGCATTG GTAAGGAGAC CAAAATCAAG GTTGTTAAAA ACAAGGTCGC TCCGCCATTT	660
GCCAGCATTG GTAAGGAGAC CARRIED	720
AAGGTAGCAG AAGTTGAAAT CATGTATGGG GAAGGTATTT CTCGTACAGG GGAGCTTGTG	
AAAATTGCTT CTGATTTGGA CATTATCCAA AAAGCAGGTG CTTGGTTCTC TTATAATGGT	780
GAGAAGATTG GCCAAGGTTC TGAAAATGCT AAGCGTTATT TGGCCGATCA TCCACAATTG	840
TTTGATGARA TCGACCGTAA AGTACGTGTT AAATTTGGTT TGCTTGAAGA AAGCGAAGAA	900
GAATCTGCTA TGGCAGTAGC ATCAGAAGAA ACCGATGATC TTGCTTTAGA TTTAGATAAT	960
GGTATTGARA TTGARGATTA A	981
(2) INFORMATION FOR SEQ ID NO: 36:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 312 base pairs (B) TYPE: nucleic acid	
(C) STRANDEDNESS: double	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE:	
(vi) ORIGINAL SOURCE:  (A) ORGANISM: Streptococcus salivarius	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:	
GCGTATGCAC GAGCTCTAGG TGTTAATATC GATGAGCTTC TTTTGTCGCA GCCTGATTCT	60
GGTGAGCAAG GTCTCGAAAT TGCAGGTAAG CTGATTGACT CTGGTGCAGT GGATTTAGTT	120
GTTGTTGACT CAGTTGCGGC CTTCGTACCA CGTGCAGAAA TTGATGGAGA TAGTGGTGAC	180
AGTCATGTAG GACTTCAAGC GCGTATGATG AGTCAAGCCA TGCGTAAACT TTCTGCATCT	240
ATTAATAAAA CAAAAACGAT TGCTATCTTT ATTAACCAGT TGCGTGAAAA AGTTGGTATC	300
ATGTTTGGTA AC	312
(2) INFORMATION FOR SEQ ID NO: 37:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:	
CTATGTGGCG CGGTATTATC	20

(2) INFORMATION FOR SEQ ID NO: 38:

```
(i) SEQUENCE CHARACTERISTICS:
                                            (A) LENGTH: 20 base pairs
                                            (B) TYPE: nucleic acid
                                           (C) STRANDEDNESS: single
                                           (D) TOPOLOGY: linear
                          (ii) MOLECULE TYPE: DNA (genomic)
                         (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:
            CGCAGTGTTA TCACTCATGG
                                                                                                                                                                                                                           20
            (2) INFORMATION FOR SEQ ID NO: 39:
                           (i) SEQUENCE CHARACTERISTICS:
                                         (A) LENGTH: 20 base pairs
                                         (B) TYPE: nucleic acid
                                         (C) STRANDEDNESS: single
                                         (D) TOPOLOGY: linear
                       (ii) MOLECULE TYPE: DNA (genomic)
                       (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:
          CTGAATGAAG CCATACCAAA
                                                                                                                                                                                                                         20
          (2) INFORMATION FOR SEQ ID NO: 40:
                         (i) SEQUENCE CHARACTERISTICS:
                                       (A) LENGTH: 20 base pairs
                                       (B) TYPE: nucleic acid
                                       (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
                                                                                                                         the state of the second of the
                    (ii) MOLECULE TYPE: DNA (genomic)
                    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:
        ATCAGCAATA AACCAGCCAG
                                                                                                                                                                                                                      20
       (2) INFORMATION FOR SEQ ID NO: 41:
                       (i) SEQUENCE CHARACTERISTICS:
                               (A) LENGTH: 20 base pairs
                                     (B) TYPE: nucleic acid
                               (C) STRANDEDNESS: single
                                    (D) TOPOLOGY: linear
                  (ii) MOLECULE TYPE: DNA (genomic)
                  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:
     TTACCATGAG CGATAACAGC
                                                                                                                                                                                                                    20
```

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

	(A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDENESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:	
CTC	ATTCAGT TCCGTTTCCC	20
(2)	INFORMATION FOR SEQ ID NO: 43:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:	
CAG	CTGCTGC AGTGGATGGT	20
(2)	INFORMATION FOR SEQ ID NO: 44:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:	
CGC	CTGCTT TGTTATTCGG	20
(2)	INFORMATION FOR SEQ ID NO: 45:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:	
TAC	ECCAACA TCGTGGAAAG	20
(2)	INFORMATION FOR SEQ ID NO: 46:	

- 90 -

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:	
TTGAATTTGG CTTCTTCGGT	20
(2) INFORMATION FOR SEQ ID NO: 47:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:	
GGGATACAGA AACGGGACAT	20
(2) INFORMATION FOR SEQ ID NO: 48:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:	
TAAATCTTTT TCAGGCAGCG	20
(2) INFORMATION FOR SEQ ID NO: 49:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 25 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:	
GATGGTTTGA AGGGTTTATT ATAAG	25
(2) INFORMATION FOR SEQ ID NO: 50:	

	(A) LEWOTH: 25 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:	
AAT.	TTAGTGT GTTTAGAATG GTGAT	25
(2)	INFORMATION FOR SEQ ID NO: 51:	
	(i) SEQUENCE CHARACTERISTICS: (A) LEMOTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:	
ACT.	CAACAC CTGCTGCTTT C	21
(2)	INFORMATION FOR SEQ ID NO: 52:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:	
TGA	CCACTTT TATCAGCAAC C	21
(2)	INFORMATION FOR SEQ ID NO: 53:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:	
GGC.	AATAGTT GAAATGCTCG	20
(2)	INFORMATION FOR SEQ ID NO: 54:	

- 92 -

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:	
CAGCTGTTAC AACGGACTGG 2	0
(2) INFORMATION FOR SEQ ID NO: 55:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:	
TOTATGATCT CGCAGTCTCC 2	0
(2) INFORMATION FOR SEQ ID NO: 56:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	-
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:	
ATCGTCACCG TAATCTGCTT 2	0
(2) INFORMATION FOR SEQ ID NO: 57:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:	
CATTCTCGAT TGCTTTGCTA 2	0
(2) INFORMATION FOR SEQ ID NO: 58:	

(i) SEQUENCE CHARACTERISTICS: (A) LENOTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:	
CCGAAATGCT TCTCAAGATA	20
(2) INFORMATION FOR SEQ ID NO: 59:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:	
CTGGATTATG GCTACGGAGT	20
(2) INFORMATION FOR SEQ ID NO: 60:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	e e e e e e e e e e e e e e e e e e e
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:	
AGCAGTGTGA TGGTATCCAG	20
(2) INFORMATION FOR SEQ ID NO: 61:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:	
GACTCTTGAT GAAGTGCTGG	20

(2) INFORMATION FOR SEQ ID NO: 62:

- 94 -

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:	
CTGGTCTATT CCTCGCACTC	
(2) INFORMATION FOR SEQ ID NO: 63:	20
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: Nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:	
TATGAGAAGG CAGGATTCGT	20
(2) INFORMATION FOR SEQ ID NO: 64:	20
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:	
GCTTTCTCTC GAAGGCTTGT	20
(2) INFORMATION FOR SEQ ID NO: 65:	20
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDMESS: single (D) TOFOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:	
GAGTTGCTGT TCAATGATCC	2.2
(2) INFORMATION FOR SEQ ID NO: 66:	20

(i) SEQUENCE CHARACTERISTICS:

	(A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:	
GTGT	TTTGAAC CATGTACACG	20
(2)	INFORMATION FOR SEQ ID NO: 67:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:	
TGT	AGAGGTC TAGCCCGTGT	20
(2)	INFORMATION FOR SEQ ID NO: 68:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:	
ACG	GGGATAA CGACTGTATG	20
(2)	INFORMATION FOR SEQ ID NO: 69:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
	(ii) MOLECULE TYPE: DNA (genomic)	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:	
ATA	AAGATGA TAGGCCGGTG	20
(2)	INFORMATION FOR SEQ ID NO: 70:	

- 96 -

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDMESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:	
TGCTGTCATA TTGTCTTGCC	20
(2) INFORMATION FOR SEQ ID NO: 71:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRADEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:	
ATTATCTTCG GCGGTTGCTC	20
(2) INFORMATION FOR SEQ ID NO: 72:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:	
GACTATCGGC TTCCCATTCC	20
(2) INFORMATION FOR SEQ ID NO: 73:	20
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:	
CGATAGAAGC AGCAGGACAA	20
(2) INFORMATION FOR SEO ID NO. 74.	

- 97 -

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:	20
CTGATGGATG CGGAAGATAC	20
(2) INFORMATION FOR SEQ ID NO: 75:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:	
GCCTTATGTA TGAACAAATG G	21
(2) INFORMATION FOR SEQ ID NO: 76:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	· ca.t. at a w
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:	
GTGACTTTWG TGATCCCTTT TGA	23
(2) INFORMATION FOR SEQ ID NO: 77:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:	
TCCAATCATT GCACAAAATC	20

(2) INFORMATION FOR SEQ ID NO: 78:

- 98 -

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDMESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:	
AATTCCCTCT ATTTGGTGGT	20
(2) INFORMATION FOR SEQ ID NO: 79:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:	
TCCCAAGCCA GTAAAGCTAA	20
(2) INFORMATION FOR SEQ ID NO: 80:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	·int
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:	
TGGTTTTTCA ACTTCTTCCA	20
(2) INFORMATION FOR SEQ ID NO: 81:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDMESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:	
TCATAGAATG GATGGCTCAA	20
(2) INFORMATION FOR SEC ID NO. 92.	

(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 20 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
(D) TOPOLOGI: Illiear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:	
	20
AGCTACTATT GCACCATCCC	
(2) INFORMATION FOR SEQ ID NO: 83:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 22 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:	
CAATAAGGGC ATACCAAAAA TC	22
(2) INFORMATION FOR SEQ ID NO: 84:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 22 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	51 11 11 11 11 11
(D) TOPOLOGY: linear	
4 1 1	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:	
(XI) SEQUENCE DESCRIPTION: DDg	
CCTTAACATT TGTGGCATTA TC	22
(2) INFORMATION FOR SEQ ID NO: 85:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 22 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
Type (renemia)	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:	
(XI) SEGUENCE DESCRIPTIONS	
TTGGGAAGAT GAAGTTTTTA GA	22

(2) INFORMATION FOR SEQ ID NO: 86:

- 100 -

(A) LENGTH: 22 base pairs (B) TYPE: nucleic acid (C) STRANDENNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:	
CCTTTACTCC AATAATTTGG CT	22
(2) INFORMATION FOR SEQ ID NO: 87:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:	
TTTCATCTAT TCAGGATGGG	20
(2) INFORMATION FOR SEQ ID NO: 88:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDMESS: single (D) TOPOLOGY: linear	Comment of
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:	
GGAGCAACAT TCTTTGTGAC	20
(2) INFORMATION FOR SEQ ID NO: 89:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:	
TGTGCCTGAA GAAGGTATTG	20
(2) INFORMATION FOR SEO ID NO: 90.	

(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:	
CGTGTTACTT CACCACCACT	20
(2) INFORMATION FOR SEQ ID NO: 91:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 23 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDMESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:	
TATCTTATCG TTGAGAAGGG ATT	23
(2) INFORMATION FOR SEQ ID NO: 92:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 22 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	Park and as here o
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:	
CTACACTTGG CTTAGGATGA AA	22
(2) INFORMATION FOR SEQ ID NO: 93:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 24 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDMESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:	
CTATCTGATT GTTGAAGAAG GATT	24
(2) INFORMATION FOR SEQ ID NO: 94:	

- 102 -

(1) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 24 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:	
GTTTACTCTT GGTTTAGGAT GAAA	24
(2) INFORMATION FOR SEQ ID NO: 95:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:	
CTTGTTGATC ACGATAATTT CC	22
(2) INFORMATION FOR SEQ ID NO: 96:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:	
ATCTTTTAGC AAACCCGTAT TC	22
(2) INFORMATION FOR SEQ ID NO: 97:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:	
AACAGGTGAA TTATTAGCAC TTGTAAG	27
(2) INFORMATION FOR SEQ ID NO: 98:	

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:	
ATTGCTGTTA ATATTTTTG AGTTGAA	27
(2) INFORMATION FOR SEQ ID NO: 99:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 19 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:	
GTGATCGAAA TCCAGATCC	19
(2) INFORMATION FOR SEQ ID NO: 100:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 19 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDMESS: single  (D) TOPOLOGY: linear	a e Managa de Campany
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:	
ATCCTCGGTT TTCTGGAAG	19
(2) INFORMATION FOR SEQ ID NO: 101:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 19 base pairs (B) TYPE: nucleic acid (C) STRANDENDESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:	
CTGGTCATAC ATGTGATGG	19
(2) INFORMATION FOR SEQ ID NO: 102:	

- 104 -

(1) SEQUENCE CHARACTERISTICS: (A) LENGTH: 19 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:	
GATGTTACCC GAGAGCTTG	19
(2) INFORMATION FOR SEQ ID NO: 103:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDMESS: single  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:	
TTAAGCGTGC ATAATAAGCC	20
(2) INFORMATION FOR SEQ ID NO: 104:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 20 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: single  (D) TOPOLOGY: linear	neo con la la regera <u>arc</u> a e la constancia
(ii) MOLECULE TYPE: DNA (genomic)	1.50 1.000
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:	
TTGCGATTAC TTCGCCAACT	20
(2) INFORMATION FOR SEQ ID NO: 105:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:	
TTTACTAAGC TTGCCCCTTC	20
(2) INFORMATION FOR SEO ID NO. 106.	

20

29

(i)	SEOUENCE	CHARACTERISTICS	3	:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

### AAAAGGCAGC AATTATGAGC

(2) INFORMATION FOR SEQ ID NO: 107:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:9
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:12
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:15
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION: 18
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:21
      - (D) OTHER INFORMATION:/note= "n = inosine"
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

### AAYATGATNA CNGGNGCNGC NCARATGGA

TNA CNGGNGCNGC NCARATGGA

and the second of the second of the second of

- (2) INFORMATION FOR SEQ ID NO: 108:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 23 base pairs
      - (B) TYPE: nucleic acid

23

er commercial and the second

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION: 3
  - (D) OTHER INFORMATION: /note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION: 6
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:9
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (ix) FEATURE:
  - (A) NAME/KEY: misc\_feature
  - (B) LOCATION:12
  - (D) OTHER INFORMATION:/note= "n = inosine"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

#### CCNACNGTNC KNCCRCCYTC RCG

(2) INFORMATION FOR SEQ ID NO: 109:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
  - (B) TYPE: nucleic acid (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
      - (B) LOCATION:6
      - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:12
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION: 15
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature

<pre>(B) LOCATION:18 (D) OTHER INFORMATION:/note= "n = inosine"</pre>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:	
CARYTNATHG TNGCNGTNAA YAARATGGA	29
(2) INFORMATION FOR SEQ ID NO: 110:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 831 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:	
ATGAAAAACA CAATACATAT CAACTTCGCT ATTTTTTTAA TAATTGCAAA TATTATCTAC	60
AGCAGCGCCA GTGCATCAAC AGATATCTCT ACTGTTGCAT CTCCATTATT TGAAGGAACT	120
GAAGGTTGTT TTTTACTTTA CGATGCATCC ACAAACGCTG AAATTGCTCA ATTCAATAAA	180
GCAAAGTGTG CAACGCAAAT GGCACCAGAT TCAACTTTCA AGATCGCATT ATCACTTATG	240
GCATTTGATG CGGAAATAAT AGATCAGAAA ACCATATTCA AATGGGATAA AACCCCCAAA	300
GGATTGAGA TCTGGAACAG CAATCATACA CCAAAGACGT GGATGCAATT TTCTGTTGTT	360
TGGGTTTCGC AAGAAATAAC CCAAAAAATT AGATTAAATA AAATCAAGAA TTATCTCAAA	420
GATTTTGATT ATGGARATCA AGACTTCTCT GGAGATARAG ARAGARACAR CGGATTRACA	480
GAAGCATGGC TCGAAAGTAG CTTAAAAATT TCACCAGAAG AACAAATTCA ATTCCTGCGT	540
AAAATTATTA ATCACAATCT CCCAGTTAAA AACTCAGCCA TAGAAAACAC CATAGAGAAC	600
ATGTATCTAC AAGATCTGGA TAATAGTACA AAACTGTATG GGAAAACTGG TGCAGGATTC	660
ACAGCAAATA GAACCTTACA AAACGGATGG TTTGAAGGGT TTATTATAAG CAAATCAGGA	720
CATARATATG TTTTTGTGTC CGCACTTACA GGARACTTGG GGTCGAATTT AACATCAAGC	780
ATAAAAGCCA AGAAAAATGC GATCACCATT CTAAACACAC TAAATTTATA A	831
(2) INFORMATION FOR SEQ ID NO: 111:	
<ul><li>(i) SEQUENCE CHARACTERISTICS:</li><li>(A) LENGTH: 846 base pairs</li><li>(B) TypE: nucleic acid</li></ul>	

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SE	QUENCE	DESCRIPTION:	SEO	ID	NO:	331
---------	--------	--------------	-----	----	-----	-----

TTGAAAAGT TAATATTTTT AATTGTAATT GCTTTAGTTT TAAGTGCATG TAATTCAAAC	60
AGTTCACATG CCAAAGAGTT AAATGATTTA GAAAAAAAT ATAATGCTCA TATTGGTGTT	120
TATGCTTTAG ATACTAAAAG TGGTAAGGAA GTAAAATTTA ATTCAGATAA GAGATTTGCC	180
TATGCTTCAA CTTCAAAAGC GATAAATAGT GCTATTTTGT TAGAACAAGT ACCTTATAAT	240
AAGTTAAATA AAAAAGTACA TATTAACAAA GATGATATAG TTGCTTATTC TCCTATTTTA	300
GAAAAATATG TAGGAAAAGA TATCACTTTA AAAGCACTTA TTGAGGCTTC AATGACATAT	360
AGTGATAATA CAGCAAACAA TAAAATTATA AAAGAAATCG GTGGAATCAA AAAAGTTAAA	420
CAACGTCTAA AAGAACTAGG AGATAAAGTA ACAAATCCAG TTAGATATGA GATAGAATTA	480
AATTACTATT CACCAAAGAG CAAAAAAGAT ACTTCAACAC CTGCTGCTTT CGGTAAGACT	540
TTAAATAAAC TTATCGCAAA TGGAAAATTA AGCAAAGAAA ACAAAAAATT CTTACTTGAT	600
TTAATGTTAA ATAATAAAAG CGGAGATACT TTAATTAAAG ACGGTGTTCC AAAAGACTAT	660
AAGGTTGCTG ATAAAAGTGG TCAAGCAATA ACATATGCTT CTAGAAATGA TGTTGCTTTT	720
GTTTATCCTA AGGGCCAATC TGAACCTATT GTTTTAGTCA TTTTTACGAA TAAAGACAAT	780
AAAAGTGATA AGCCAAATGA TAAGTTGATA AGTGAAACCG CCAAGAGTGT AATGAAGGAA	840
TTTTAA	846
(2) INFORMATION FOR SEQ. ID NO: 112:	St. V. wilder Time.

- - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 555 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

ATGTCCGCGA GCACCCCCC CATAACTCTT CGCCTCATGA CCGAGCGCGA CCTGCCGATG 60 CTCCATGACT GGCTCAACCG GCCGCACATC GTTGAGTGGT GGGGTGGCGA CGAAGAGCGA 120 CCGACTCTTG ATGAAGTGCT GGAACACTAC CTGCCCAGAG CGATGGCGGA AGAGTCCGTA 180 ACACCGTACA TCGCAATGCT GGGCGAGGAA CCGATCGGCT ATGCTCAGTC GTACGTCGCG 240 CTCGGAAGCG GTGATGGCTG GTGGGAAGAT GAAACTGATC CAGGAGTGCG AGGAATAGAC 300 CAGTCTCTGG CTGACCCGAC ACAGTTGAAC AAAGGCCTAG GAACAAGGCT TGTCCGCGCT 360

CTCGTTGAAC TACTGTTCTC GGACCCCACC GTGACGAAGA TTCAGACCGA CCCGACTCCG	420
AACAACCATC GAGCCATACG CTGCTATGAG AAGGCAGGAT TCGTGCGGGA GAAGATCATC	480
ACCACGCCTG ACGGGCCGGC GGTTTACATG GTTCAAACAC GACAAGCCTT CGAGAGAAAG	540
CGCGGTGTTG CCTAA	555
(2) INFORMATION FOR SEQ ID NO: 113:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 732 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:	
ATGAACCAGA AAAACCCTAA AGACACGCAA AATTTTATTA CTTCTAAAAA GCATGTAAAA	60
GARATATTGA ATCACACGAA TATCAGTAAA CAAGACAACG TAATAGAAAT CGGATCAGGA	120
AAAGGACATT TTACCAAAGA GCTAGTCAAA ATGAGTCGAT CAGTTACTGC TATAGAAATT	180
GATGGAGGCT TATGTCAAGT GACTAAAGAA GCGGTAAACC CCTCTGAGAA TATAAAAGTG	240
ATTCAAACGG ATATTCTAAA ATTTTCCTTC CCAAAACATA TAAACTATAA GATATATGGT	300
ARTATTCCTT ATAACATCAG TACGGATATT GTCAAAAGAA TTACCTTTGA AAGTCAGGCT	360
AAATATAGCT ATCTTATCGT TGAGAAGGGA TTTGCGAAAA GATTGCAAAA TCTGCAACGA	420 .
GCTTTGGGTT TACTATTAAT GGTGGAGATG GATATAAAAA TGCTCAAAAA AGTACCACCA	480
CTATATTTC ATCCTAAGCC AAGTGTAGAC TCTGTATTGA TTGTTCTTGA ACGACATCAA	540
CCATTGATTT CAAAGAAGGA CTACAAAAAG TATCGATCTT TTGTTTATAA GTGGGTAAAC	600
CGTGAATATC GTGTTCTTTT CACTAAAAAC CAATTCCGAC AGGCTTTGAA GCATGCAAAT	660
GTCACTAATA TTAATAAACT ATCGAAGGAA CAATTTCTTT CTATTTTCAA TAGTTACAAA	720
TTGTTTCACT AA	732
- 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	

- (2) INFORMATION FOR SEQ ID NO: 114:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 738 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear
  - •
  - (ii) MOLECULE TYPE: DNA (genomic)

(xi)	SEQUENCE	DESCRIPTION:	SEQ	ID	NO:	114
------	----------	--------------	-----	----	-----	-----

ATGAACAAA	מדמממתמדם ב	TTCTCAAAA				
					ACTCAACCAA	60
ATAATAAAA	AATTGAATTT	AAAAGAAACC	GATACCGTTT	ACGAAATTGG	AACAGGTAAA	120
GGGCATTTA	CGACGAAACT	GGCTAAAATA	AGTAAACAGG	TAACGTCTAT	TGAATTAGAC	180
AGTCATCTAT	TCAACTTATC	GTCAGAAAA	TTAAAATCGA	ATACTCGTGT	CACTTTAATT	240
CACCAAGATA	TTCTACAGTT	TCAATTCCCT	AACAAACAGA	GGTATAAAAT	TGTTGGGAAT	300
ATTCCTTACC	ATTTAAGCAC	ACAAATTATT	AAAAAGTGG	TTTTTGAAAG	CCATGCGTCT	360
GACATCTATC	TGATTGTTGA	AGAAGGATTC	TACAAGCGTA	CCTTGGATAT	TCACCGAACA	420
CTAGGGTTGC	TCTTGCACAC	TCAAGTCTCG	ATTCAGCAAT	TGCTTAAGCT	GCCAGCGGAA	480
TGCTTTCATC	CTAAACCAAG	AGTAAACAGT	GTCTTAATAA	AACTTACCCG	CCATACCACA	540
GATGTTCCAG	ATAAATATTG	GAAGCTATAT	ACGTACTTTG	TTTCAAAATG	GGTCAATCGA	600
GAATATCGTC	AACTGTTTAC	TAAAAATCAG	TTTCATCAAG	CAATGAAACA	CGCCAAAGTA	660
ACAATTTAA	GTACCGTTAC	TTATGAGCAA	GTATTGTCTA	TTTTTAATAG	TTATCTATTA	720
TTAACGGGA	GGAAATAA					738

- (2) INFORMATION FOR SEQ ID NO: 115:
  - (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 735 base pairs (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

ATGAACGAGA AAAATATAAA ACACAGTCAA AACTTTATTA CTTCAAAACA TAATATAGAT 60 AAAATAATGA CAAATATAAG ATTAAATGAA CATGATAATA TCTTTGAAAT CGGCTCAGGA 120 AAAGGGCATT TTACCCTTGA ATTAGTACAG AGGTGTAATT TCGTAACTGC CATTGAAATA 180 GACCATAAAT TATGCAAAAC TACAGAAAAT AAACTTGTTG ATCACGATAA TTTCCAAGTT 240 TTAAACAAGG ATATATTGCA GTTTAAATTT CCTAAAAACC AATCCTATAA AATATTTGGT 300 AATATACCTT ATAACATAAG TACGGATATA ATACGCAAAA TTGTTTTTGA TAGTATAGCT 360 GATGAGATTT ATTTAATCGT GGAATACGGG TTTGCTAAAA GATTATTAAA TACAAAACGC 420 TCATTGGCAT TATTTTAAT GGCAGAAGTT GATATTTCTA TATTAAGTAT GGTTCCAAGA 480

GAATATTTC ATCCTAAACC TAGAGTGAAT AGCTCACTTA TCAGATTAAA TAGAAAAAAA	540
TCAAGAATAT CACACAAAGA TAAACAGAAG TATAATTATT TCGTTATGAA ATGGGTTAAC	600
AAAGAATACA AGAAAATATT TACAAAAAAT CAATTTAACA ATTCCTTAAA ACATGCAGGA	660
ATTGACGATT TAAACAATAT TAGCTTTGAA CAATTCTTAT CTCTTTTCAA TAGCTATAAA	720
TTATTTAATA AGTAA	735
(2) INFORMATION FOR SEQ ID NO: 116:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1029 base pairs (B) TYPE: nucleic acid (C) STRANDEDMESS: double (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:	

ATGAATAAAA TAAAAGTCGC AATTATCTTC GGCGGTTGCT CGGAGGAACA TGATGTGTCG 60 GTAAAATCCG CAATAGAAAT TGCTGCGAAC ATTAATACTG AAAAATTCGA TCCGCACTAC 120 ATCGGAATTA CAAAAAACGG CGTATGGAAG CTATGCAAGA AGCCATGTAC GGAATGGGAA 180 GCCGATAGTC TCCCCGCCAT ATTCTCCCCG GATAGGAAAA CGCATGGTCT GCTTGTCATG 240 AAAGAAAGAG AATACGAAAC TCGGCGTATT GACGTGGCTT TCCCGGTTTT GCATGGCAAA 300 TGCGGGGAGG ATGGTGCGAT ACAGGGTCTG TTTGAATTGT CTGGTATCCC CTATGTAGGC 360 TGCGATATTC AAAGCTCCGC AGCTTGCATG GACAAATCAC TGGCCTACAT TCTTACAAAA 420 AATGCGGGCA TCGCCGTCCC CGAATTTCAA ATGATTGAAA AAGGTGACAA ACCGGAGGCG 480 AGGACGCTTA CCTACCCTGT CTTTGTGAAG CCGGCACGGT CAGGTTCGTC CTTTGGCGTA 540 ACCAAAGTAA ACAGTACGGA AGAACTAAAC GCTGCGATAG AAGCAGCAGG ACAATATGAT 600 GGAAAAATCT TAATTGAGCA AGCGATTTCG GGCTGTGAGG TCGGCTGCGC GGTCATGGGA 660 AACGAGGATG ATTTGATTGT CGGCGAAGTG GATCAAATCC GGTTGAGCCA CGGTATCTTC 720 CGCATCCATC AGGAAAACGA GCCGGAAAAA GGCTCAGAGA ATGCGATGAT TATCGTTCCA 780 GCAGACATTC CGGTCGAGGA ACGAAATCGG GTGCAAGAAA CGGCAAAGAA AGTATATCGG 840 GTGCTTGGAT GCAGAGGGCT TGCTCGTGTT GATCTTTTTT TGCAGGAGGA TGGCGGCATC 900 GTTCTAAACG AGGTCAATAC CCTGCCCGGT TTTACATCGT ACAGCCGCTA TCCACGCATG 960 GCGGCTGCCG CAGGAATCAC GCTTCCCGCA CTAATTGACA GCCTGATTAC ATTGGCGATA 1020

- 112 -

CA	~~	9	TGA

1029

- (2) INFORMATION FOR SEQ ID NO: 117:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 1031 base pairs
      - (B) TYPE: nucleic acid
      - (C) STRANDEDNESS: double
      - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

					T GTCACTAACC	60
	A GTGTGATCCA					120
	A CAATGGATTG					180
ACTTGGCTA	G AAGATCACAA	AAACTGTCAC	CAGCTGACTT	TTTCTAGCC	AGGATTTATA	240
	A AACGAATCGT					300
GAGGATGGC	r gtatccaagg	ACTGCTTGAA	CTAATGAACC	TGCCTTATGT	TGGTTGCCAT	360
GTCGCTGCC	CCGCATTATG	TATGAACAAA	TGGCTCTTGC	ATCAACTTGC	TGATACCATG	420
GGAATCGCTZ	GTGCTCCCAC	TTTGCTTTTA	TCCCGCTATG	AAAACGATCC	TGCCACAATC	480
GATCGTTTTA	TTCAAGACCA	TGGATTCCCG	ATCTTTATCA	AGCCGAATGA	AGCCGGTTCT	540
TCAAAAGGGA	TCACAAAAGT	AACTGACAAA	ACAGCGCTCC	AATCTGCATT	AACGACTGCT	600
TTTGCTTACG	GTTCTACTGT	GTTGATCCAA	AAGGCGATAG	CGGGTATTGA	AATTGGCTGC	660
GGCATCTTAG	GAAATGAGCA	ATTGACGATT	GGTGCTTGTG	ATGCGATTTC	TCTTGTCGAC	720
GGTTTTTTTG	ATTTTGAAGA	GAAATACCAA	TTAATCAGCG	CCACGATCAC	TGTCCCAGCA	780
CCATTGCCTC	TCGCGCTTGA	ATCACAGATC	AAGGAGCAGG	CACAGCTGCT	TTATCGAAAC	840
TTGGGATTGA	CGGGTCTGGC :	CGAATCGAT	TTTTTCGTCA	CCAATCAAGG	AGCGATTTAT	900
TTAAACGAAA	TCAACACCAT (	GCCGGGATTT 1	ACTGGGCACT	CCCGCTACCC	AGCTATGATG	960
	GGTTATCCTA (					1020
GACAAACGAT						1031

- (2) INFORMATION FOR SEQ ID NO: 118:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 809 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double

(D)	TOPOLOGY:	linear
-----	-----------	--------

(ii)	MOLECULE	TYPE:	DNA	(genomic)
------	----------	-------	-----	-----------

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Abiotrophia adiacens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

(xi) SEQUENCE DESCRIPTION: 522 -		
TGGTGCTATC TTAGTAGTAT CTGCAGCTGA TGGTCCAATG CCTCAAACAC	GTGAACACAT	60
CTTATTATCA CGTCAAGTAG GTGTTCCTTA CATCGTTGTA TTCTTAAACA		120
GGTTGACGAT GAAGAATTAT TAGAATTAGT AGAAATGGAA GTTCGTGACT	TATTATCAGA	180
ATACGATTTC CCAGGCGATG ACACTCCAGT TGTTGCAGGT TCTGCTTTAC		240
AGGCGACGCT TCATACRAAG AAAAAATCTT AGAATTAATG GCTGCTGTTG		300
TCCAACTCCA GAACGYGACG TTGACAAACC ATTCATGATG CCAGTTGAAG	ACGTGTTCTC	360
AATCACAGGT CGTGGTACTG TTGCTACAGG TCGTGTTGAA CGTGGACAAG		420
TGACGAAGTT GAAATCGTTG GTATTTCAGA AGAAACTTCA AAAACAACTG	TAACTGGTGT	480
TGAAATGTTC CGTAAATTGT TAGACTACGC TGAAGCAGGG GATAACATTG	GTACATTATT	540
ACGTGGTGTT ACACGTGACA ACATCGAACG TGGACAAGTT CTTGCTAAAC	CAGGAACAAT	600
CACTCCACAT ACTAAATTCA AAGCTGAAGT TTACGTATTA ACTAAAGAAG	AAGGTGGACG	660
TCATACTCCA TTCTTCTCTA ACTACCGTCC TCAATTCTAC TTCCGTACAA	CAGACATCAC	720
TGGTGTTTGT GTGTTACCAG AAGGCGTTGA AATGGTAATG CCTGGTGATA	ACGTAACTAT	780
GGAAGTTGAA TTAATTCACC CAGTAGCGA		809

- (2) INFORMATION FOR SEQ ID NO: 119:
  - (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 817 base pairs
    - (B) TYPE: nucleic acid

    - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Abiotrophia defectiva
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 119:

CGGCGCGATC CTCGTTGTAT CTGCTGCTGA CGGCCCAATG CCACAAACTC GTGAACACAT CCTCTTGTCT CGTCAAGTTG GTGTTCCTTA CATCGTAGTA TTCTTGAACA AAGTTGACAT 120

was a series of the series of the series of the series of the series of

COTTONOCA						
	C GAAGAATTGC					18
	C CCAGGCGACG					24
AGGCGACGC	T AACTACGAAG	CTAAAGTTTT	AGAATTGATG	GAACAAGTTG	ATGCTTACAT	300
TCCAGAACC	A GAACGTGACA	CTGACAAGCC	ATTCATGATG	CCAGTCGAAG	ACGTATTCTC	360
TATCACTGG	T CGTGGTACTG	TTGCAACTGG	TCGTGTTGAA	CGTGGTCAAG	TTCGCGTTGG	420
	T GAAATCGTTG					480
	CGTAAGTTAT					540
	ACTCGTGACC					600
	ACTAAGTTCG					660
	TTCTTCTCTA					720
	ACTTTACCAG					
	TTGATCCACC (			Jones 2	ACGIACAAAT	780
	ATION FOR SE					817
	TOR SE	2 ID NO: 12	0:			

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 754 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Candida albicans
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 120:

CTCTGTCAAA TGGGACAAAA ACAGATTTGA AGAAATCATC AAGGAAACCT CCAACTTCGT 60 CAAGAAGGTT GGTTACAACC CAAAGACTGT TCCATTCGTT CCAATCTCTG GTTGGAATGG 120 TGACAACWTG ATTGAASCAT CCACCAACTG TCCATGGTAC AAGGGTTGGG AAAAGGAAAC 180 CAAATCCGGT AAAGTTACTG GTAAGACCTT GTTAGAAGCT ATTGACGCTA TTGAACCACC 240 AACCAGACCA ACCGACAAAC CATTGAGATT GCCATTRCAA GATGTTTACA AGATCGGTGG 300 TATTGGTACT GTGCCAGTCG GTAGAGTTGA AACTGGTATC ATCAAAGCCG GTATGGTWGT 360 TACTTTCGCC CCAGCTGGTG TTACCACTGA AGTCAARTCC GTTGAAATGC ATCACGAACA 420 ATTGGCTGAA GGTGTTCCAG GTGACAATGT TRGTTTCAAC GTTAAGAACR TTTCCGTTAA 480 AGAAATTAGA AGAGGTAACG TTTGTGGTGA CTCCAAGAAC GATCCACCAA AGGGTTGTGA 540

CTCTTTCAAT GCCCAAGTCA TIGTTTTGAA CCATCCAGGT CAAATCTCTG CTGGTTACTC	600
TCCAGTCTTG GATTGTCACR CTGCCCACAT TGCTTGTAAA TTCGACRCTT TGGTTGAAAA	660
GATTGACAGA AGAACTGGTA AGRAATTGGA AGAAAATCCA AAATTCGTCA AATCCGGTGA	720
TGCTGCTATC GTCAAGATGG TCCCAACCAA ACCA	754
(2) INFORMATION FOR SEQ ID NO: 121:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 753 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
<ul><li>(vi) ORIGINAL SOURCE:</li><li>(A) ORGANISM: Candida glabrata</li></ul>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 121:	
TCTGTCAAGT GGGATGAATC CAGATTCGCT GAAATCGTTA AGGAAACCTC CAACTTCATC	60
AAGAAGGTCG GTTACAACCC AAAGACTGTT CCATTCGTCC CAATCTCTGG TTGGAACGGT	120
GACAACATGA TTGAAGCCAC CACCAACGCT TCCTGGTACA AGGGTTGGGA AAAGGAAACC	180
AAGGCTGGTG TCGTCAAGGG TAAGACCTTG TTGGAAGCCA TTGACGCTAT CGAACCACCA	240
ACCAGACCAA CTGACAAGCC ATTGAGATTG CCATTGCAAG ATGTCTACAA GATCGGTGGT	300
ATCGGTACGG TGCCAGTCGG TAGAGTCGAA ACCGGTGTCA TCAAGCCAGG TATGGTTGTT	360
ACCTTCGCCC CAGCTGGTGT TACCACTGAA GTCAAGTCCG TTGAAATGCA CCACGAACAA	420
TTGACTGAAG GTTTGCCAGG TGACAACGTT GGTTTCAACG TTAAGAACGT TTCCGTTAAG	480
GANATCAGAN GAGGTANTGT CTGTGGTGAC TCCANGANCG ACCCACCANA GGCTGCTGCT	540
TCTTTCAACG CTACCGTCAT TGTCTTGAAC CACCCAGGTC AAATCTCTGC TGGTTACTCT	600
CCAGTTTTGG ACTGTCACAC CGCCCACATT GCTTGTAAGT TCGAAGAATT GTTGGAAAAG	660
AACGACAGAA GATCCGGTAA GAAGTTGGAA GACTCTCCAA AGTTCTTGAA GTCCGGTGAC	720
GCTGCTTTGG TTAAGTTCGT TCCATCCAAG CCA	753
GCIGCIIIGG IIMAGIIGG	

(2) INFORMATION FOR SEQ ID NO: 122:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 752 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii)	MOLECULE	TYPE:	DNA	(genomic)	,
------	----------	-------	-----	-----------	---

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Candida krusei
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 122:

CCGTTAAGTG GGATGAAAAC AGATTTGAAG AAATTGTCAA GGAAACCCAA AACTTCATCA 60 AGAAGGTTGG TTACAACCCA AAGACTGTTC CATTCGTTCC AATCTCTGGT TGGAATGGTG 120 ACAACATGAT TGAAGCATCC ACCAACTGTC CATGGTACAA GGGTTGGACT AAGGAAACCA 180 AGGCAGGTGT TGTTAAGGGT AAGACCTTAT TAGAAGCAAT CGATGCTATT GAACCACCTG 240 TCAGACCAAC CGAAAAGCCA TTAAGATTAC CATTACAAGA TGTTTACAAG ATTGGTGGTA 300 TTGGTACTGT GCCAGTCGGT AGAGTCGAAA CCGGTGTCAT TAAGCCAGGT ATGGTTGTCA 360 CTTTTGCTCC AGCAGGTGTC ACCACCGAAG TCAAATCCGT TGAAATGCAC CATGAACAAT 420 TAGAACAAGG TGTTCCAGGT GATAACGTTG GTTTCAACGT TAAGAACGTY TCTGTCAAGG 480 ATATCAAGAG AGGTAACGTT TGTGGTGACT CCAAGAACGA CCCACCAATG GGTGCAGCTT 540 CTTTCAATGC TCAAGTCATT GTCTTGAACC ACCCTGGTCA AATTTCCGCT GGTTACTCTC 600 CAGTCTTGGA TTGTCACACT GCCCACATTG CATGTAAGTT CGACGAATTA ATCGAAAAGA 660 TTGACAGAAG AACTGGTAAG TCTGTTGAAG ACCATCCAAA GTCYGTCAAG TCTGGTGATG 720 CAGCTATCGT CAAGATGGTC CCAACCAAGC CA 752 and the second residue of the second residue to the second 

- (2) INFORMATION FOR SEQ ID NO: 123:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 754 base pairs
    - (B) TYPE: nucleic acid
      - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Candida parapsilosis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 123:

CTCAGTCAAA TGGGACAAGA RCAGATACGA AGAAATTGTC AAGGAAACTT CCAACTTCGT 60 CAAGAAGGTT GGTTACAACC CTAAAGCTGT CCCATTCGTC CCAATCTCTG GTTGGAACGG 120 TGACAATATG ATTGAACCAT CAACCAACTG TCCATGGTAC AAGGGTTGGG AAAAGGAAAC 180 TAAAGCTGGT AAGGTTACCG GTAAGACCTT GTTGGAAGCT ATCGATGCTA TCGARCCACC 240

240

300

360

420

480

540

600

660

720

AACCAGACCA ACTGACAAGC CATTGAGATT GCCATTGCAA GATGTCTACA AGATTGGTGG	300
TATTGGAACT GTGCCAGTTG GTAGAGTTGA AACCGGTATC ATCAAGGCTG GTATGGTTGT	360
TACTTTTGCC CCAGCTGGTG TTACCACTGA AGTCAAGTCC GTTGAAATGC ACCACGAACA	420
ATTGACTGAA GGTGTCCCAG GTGACAATGT TGGTTTCAAC GTCAAGAACG TTTCAGTTAA	480
GGAAATCAGA AGAGGTAACG TYTGTGGTGA CTCCAAGAAC GATCCACCAA AGGGATGTGA	540
YTCCTTCAAT GCTCAAGTTA TTGTCTTGAA CCACCCAGGT CAAATCTCTG CTGGTTACTC	600
ACCAGTCTTG GATTGTCACA CTGCCCACAT TGCTTGTAAA TTCGACACTT TGATTGAAAA	660
GATTGACAGA AGAACCGGTA AGAAATTGGA AGWTGAACCA AAATTCATCA AGTCCGGTGA	720
TGCTGCYATC GTCAAGATGG TCCCAACCAA GCCA	754
(2) INFORMATION FOR SEQ ID NO: 124:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 753 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear  (ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE:  (A) ORGANISM: Candida tropicalis	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 124:	
TCTGTTAAAT GGGACAARAA CAGATTTGAA GAAATTATCA AGGAAACYTC TAACTTCGTC	60
AAGAAGGTTG GTTACAACCC TAAGGCTGTT CCATTCGTTC CAATCTCWGG TTGGAATGGT	120
GACAACATGA TTGAAGCTTC TACCAACTGT CCATGGTACA AGGGTTGGGA AAAAGAAACC	180

AAGGCTGGTA AGGTTACCGG TAAGACTTTG TTGGAAGCCA TTGATGCTAT TGAACCACCT

TCAAGACCAA CTGACAAGCC ATTGAGATTG CCATTGCAAG ATGTTTACAA GATTGGTGGT

ATTGGTACTG TGCCAGTCGG TAGAGTTGAA ACTGGTGTCA TCAAAGCCGG TATGGTTGTT

ACTITYGCCC CAGCIGGIGI TACCACIGAA GICAAATCCG TYGAAATGCA CCACGAACAA

TTGGCTGAAG GTGTCCCAGG TGACAATGTT GGTTTCAACG TTAAGAACGT TTCTGTTAAA

GAAATTAGAA GAGGTAACGT TTGTGGTGAC TCCAAGAACG ATCCACCAAA GGGTTGTGAC

TCTTTCAACG CTCAAGTTAT TGTCTTGAAC CACCCAGGTC AAATYTCTGC TGGTTACTCT

CCAGTCTTGG ATTGTCACAC TGCTCATATT GCTTGTAAAT TCGACACCTT GGTTGAAAAG

ATTGACAGAA GAACTGGTAA GAAATTGGAA GAAAATCCAA AATTCGTCAA ATCCGGTGAT

GCTGCTATTG TCAAGATGGT TCCAACCAAA CC	CZ
-------------------------------------	----

(2) INFORMATION FOR SEQ ID NO: 125:

753

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 814 base pairs
    - (B) TYPE: nucleic acid (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Corynebacterium accolens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 125:

CGGCGCTATC	CTCCTTCTTC	OMCON NOOCH				
					GCGAGCACGT	60
TCTGCTTGCT	CGCCAGGTTG	GCGTTCCTTA	CATCCTCGTT	GCACTGAACA	AGTGCGACAT	120
GGTTGATGAT	GAGGAAATCA	TCGAGCTCGT	GGAGATGGAG	ATCTCCGAGC	TGCTCGCAGA	180
GCAGGACTAC	GATGAGGAAG	CTCCTATCGT	TCACATCTCC	GCTCTGAAGG	CACTCGAGGG	240
TGACGAGAAG	TGGGTACAGT	CCATCGTTGA	CCTGATGGAT	GCCTGCGACA	ACTCCATCCC	300
TGATCCGGAG	CGCGCTACCG	ATCAGCCGTT	CTTGATGCCT	ATCGAGGACA	TCTTCACCAT	360
TACCGGCCGC	GGTACCGTTG	TTACCGGCCG	TGTTGAGCGT	GGTCGTCTGA	ACGTCAACGA	420
GGACGTTGAG	ATCATCGGTA	TCCAGGAGAA	GTCCCAGAAC		CCGGTATCGA	480
GATGTTCCGC	AAGATGATGG	ACTACACCGA	GGCTGGCGAC	AACTGTGGTC	TGCTTCTGCG	540
TGGTACCAAG	CGTGAGGACG	TTGAGCGTGG	CCAGGTTGTT	ATCAAGCCGG	GCGCTTACAC	600
CCCTCACACC	AAGTTCGAGG	GTTCCGTCTA	CGTCCTGAAG	AAGGAAGAGG	GCGGCCGCCA	660
CACCCCGYTC	ATGAACAACT	ACCGTCCTCA	GTTCTACTTC	CGCACCACCG	ACGTTACCGG	720
TGTTGTGAAC	CTGCCTGAGG	GCACCGAGAT	GGTTATGCCT	GGCGACAACG	TTGAGATGTC	780
FGTTGAGCTC	ATCCAGCCTG	TTGCTATGGA	CGAG			914

- (2) INFORMATION FOR SEQ ID NO: 126:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 814 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:

171	OPGANTSM:	Corvnebacterium	diphteria

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 126:	
CGGCGCAATC CTCGTTGTTG CTGCCACCGA CGGCCCAATG CCTCAGACCC GTGAGCACGT	60
TCTGCTCGCT CGCCAGGTCG GCGTTCCTTA CATCCTCGTT GCTCTGAACA AGTGCGACAT	120
GGTTGATGAT GAGGAAATCA TCGAGCTCGT CGAGATGGAG ATCCRTGAGC TGCTCGCTGA	180
GCAGGATTAC GACGAAGAGG CTCCAATCAT CCACATCTCC GCACTGAAGG CTCTTGAGGG	240
CGACGAGAAG TGGACCCAGT CCATCATCGA CCTCATGCAG GCTTGCKATG ATTCCATCCC	300
AGACCCAGAG CGTGAGACCG ACAAGCCATT CCTCATGCCT ATCGAGGACA TCTTCACCAT	360
CACCGGCCGC GGTACCGTTG TTACCGGCCG TGTTGAGCGT GGCTCCCTGA AGGTCAACGA	420
GGACGTCGAG ATCATCGGTA TCCGCGAGAA KGCTACCACC ACCACCGTTA CCGGTATCGA	480
GATGTTCCGT AAGCTTCTCG ACTACACCGA GGCTGGCGAC AACTGTGGTC TGCTTCTCCG	540
TGGCGTTAAG CGCGAAGACG TTGAGCGTGG CCAGGTTGTT GTTAAGCCAG GCGCTTACAC	600
CCCTCACACC GAGTTCGAGG GCTCTGTCTA CGTTCTGTCC AAGGACGAGG GTGGCCGCCA	660
CACCCCATTC TTCGACAACT ACCGCCCACA GTTCTACTTC CGCACCACCG ACGTTACCGG	72
TGTTGTGAAG CTTCCTGAGG GCACCGAGAT GGTCATGCCT GGCGACAACG TCGACATGTC	78
CGTCACCCTG ATCCAGCCTG TCGCTATGGA TGAG	81

- (2) INFORMATION FOR SEQ ID NO: 127:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 814 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
    - (vi) ORIGINAL SOURCE:
      - (A) ORGANISM: Corynebacterium genitalium
    - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 127:

CGGCGCCATC	CTGGTTGTTG	CTGCAACCGA	TGGCCCGATG	CCGCAGACCC	GTGAGCACGT	60
TCTGCTGGCT	CGCCAGGTTG	GCGTTCCGTA	CATCCTAGTT	GCACTGAACA	AGTGCGACAT	120
GGTTGATGAT	GAGGAGCTGC	TGGAGCTCGT	CGAGATGGAG	GTCCGCGAGC	TGCTGGCTGA	180
GCAGGACTTC	GACGAGGAAG	CACCTGTTGT	TCACATCTCC	GCACTGAAGG	CCCTGGAGGG	240
CGACGAGAAG	TGGGCTAAGC	AGATCCTGGA	GCTCATGGAG	GCTTGCGACA	ACTCCATCCC	300

GGATCCGGAG CGCGAGACCG ACAAGCCGTT CCTGATGCCG GTTGRGGACA TCTTCACCAT	360
TACCGGCCGC GGTACCGTTG TTACCGGCCG TGTTGAGCGT GGCGTCCTGA ACCTGAACGA	420
CGAGGTCGAG ATCCTGGGCA TCCGCGAGAA GTCCACCAAG ACCACCGTTA CCTCCATCGA	480
GATGTTCAAC AAGCTGCTGG ACACCGCAGA GGCTGGCGAC AACGCCGCAC TGCTGCTGCG	540
TGGCCTGAAG CGCGAAGATG TTGAGCGTGG TCAGATCGTT GCTAAGCCGG GCGAGTACAC	600
CCCGCACACC GAGTTCGAGG GCTCCGTCTA CGTTCTGTCC AAGGACGAGG GTGGCCGCCA	660
CACCCCGTTC TTCGACAACT ACCGTCCGCA GTTCTATTTC CGCACCACCG ACGTTACCGG	720
TGTTGTGAAG CTGCCGGAGG GCACCGAGAT GGTTATGCCG GGCGACAACG TTGACATGTC	780
CGTCACCCTG ATCCAGCCGG TTGCTATGGA CGAG	814
(2) INFORMATION FOR SEQ ID NO: 128:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 814 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
<ul><li>(vi) ORIGINAL SOURCE:</li><li>(A) ORGANISM: Corynebacterium jeikeium</li></ul>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 128:	
CGGCGCCATC CTGGTTGTTG CCGCAACCGA TGGCCCGATG CCGCAGACCC GCGAGCACGT	60

TCTGCTGGCY CGCCAGGTTG GCGTTCCGTA CATCCTGGTT GCACTGAACA AGTGTGACAT 120 GGTTGACGAT GAGGAGCTGC TGGAGCTCGT CGAGATGGAG GTCCGCGAGC TGCTGGCTGA 180 GCAGGACTTC GACGAGGAAG CTCCGGTTGT TCACATCTCC GCACTGAAGG CCCTGGAGGG 240 CGACGAGAAG TGGGCTAACC AGATTCTCGA GCTGATGCAG GCTTGCGACG AGTCTATCCC 300 GGATCCGGAG CGCGAGACCG ACAAGCCGTT CCTGATGCCG GTTGWGGACA TCTTCACCAT 360 TACCGGTCGC GGTACCGTTG TTACCGGCCG TGTTGAGCGT GGCATCCTGA ACCTGAACGA 420 CGAGGTTGAG ATCCTGGGTA TCCGCGAGAA GTCCCAGAAG ACCACCGTTA CCTCCATCGA 480 GATGTTCAAC AAGCTGCTGG ACACCGCAGA GGCTGGCRAC AACGCTGCAC TGCTGCTGCG 540 TGGTCTGAAG CGCGAGGACG TTGAGCGTGG CCAGATCATC GCTAAGCCGG GCGAGTACAC 600 CCCGCACACC GAGTTCGAGG GCTCCGTCTA CGTTCTGTCC AAGGACGAGG GCGGCCGCCA 660 CACCCCGTTC TTCGACAACT ACCGTCCGCA GTTCTACTTC CGCACCACCG ACGTTACCGG 720

FGTTGTGAAG CTGCCTGAGG GCACCGAGAT GGTTATGCCG GGCGACAACG TYGACATGTC	780
CGTCACCCTG ATCCAGCCGG TTGCTATGGA CGAG	814
(2) INFORMATION FOR SEQ ID NO: 129:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 748 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: double (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
<ul><li>(vi) ORIGINAL SOURCE:</li><li>(A) ORGANISM: Corynebacterium pseudodiphteriticum</li></ul>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 129:	
CGGCGCTATC TTGGTTGTTG CAGCTACCGA CGGCCCAATG CCACAGACTC GCGAGCACGT	60
TCTGCTGGCT CGCCAGGTTG GCGTTCCTTA CATCCTGGTT GCACTAAACA AGTGCGACAT	120
GGTTGACGAC GAGGAAATCC TCGAGCTCGT CGAGATGGAG ATCCGCGAAT TGCTGGCTGA	180
CCAGGAATTC GACGAAGAAG CTCCAATCGT TCACATCTCC GCAGTCGGCG CCTTGGAAGG	240
CGAAGAGAGG TGGGTTAACG CCATCGTTGA ACTGATGGAT GCTTGTGACG AGTCGATCCC	300
TGATCCAGAC CGTGCTACCG ACAAGCCATT CCTGATGCCT ATCGAGGACA TCTTCACCAT	360
TACCGGTCGT GGCACCGTTG TTACGGGTCG TGTTGAGCGT GGTTCCCTGA AGGTCAACGA	420
AGAAGTCGAG ATCATCGGCA TCAAGGAAAA GTCCCAGAAG ACCACCATCA CCGGTATCGA	480
ANTGTTCCGC AAGATGCTGG ACTACACCGA GGCCGGCGAC AACGCTGGTC TGCTGCTTCG	540
CGGTACCAAG CGTGAAGACG TTGAGCGTGG ACAGGTTATC GTTGCTCCAG GTGCTTACAG	600
CACCCACAAG AAGTTCGAAG GTTCCGTCTA CGTTCTTTCC AAGGACGAGG GCGGCCGCCA	66
CACCCCGTTC TTCGACAACT ACCGTCCTCA GTTCTACTTC CGCACCACCG ACGTTACCGG	72
TGTTGTTACC CTGCCTGAGG GCACCGAG	74
(2) INFORMATION FOR SEQ ID NO: 130:	
(i) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 813 base pairs	

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- 122 -

(A)	ORGANISM:	Corynebacterium	striatum
-----	-----------	-----------------	----------

(X1)	SEQUENCE	DESCRIPTION:	SEQ	ID	NO:	130

					GAGCACGTT	60
CTTCTGGCTC	GCCAGGTTGG	CGTTCCTTAC	ATCCTCGTTG	CACTGAACA	GTGCGACATG	120
GTTGACGACG	AGGAAATTAT	CGAGCTCGTC	GAGATGGAGA	TCCGCGAACT	GCTCGCAGAG	180
CAGGACTACG	ATGAGGAAGC	TCCGATCGTT	CACATCTCTG	CTCTGAAGGC	TCTTGAGGGC	240
GRCGAGAAGT	GGGTACAGGC	TATCGTTGAC	CTGATGCAGG	CTTGCGATGA	CTCCATCCCG	3.00
GATCCGGAGC	GCGAGCTGGA	CAAGCCGTTC	CTGATGCCAA	TCGAGGACAT	CTTCACCATC	3.60
ACCGGCCGCG	GTACCGTTGT	TACTGGCCGT	GTTGAGCGTG	GCTCCCTGAA	CGTCAACGAG	420
GACGTTGAGA	TCATCGGTAT	CCAGGACARG	TCCATCTCCA	CCACCGTTAC	CGGTATCGAG	480
ATGYTCCGCA	AGATGATGGA	CTACACCGAG	GCTGGCGACA	ACTGTGGTCT	GCTTCTGCGT	540
	GTGAAGAGGT					600
	AGTTCGAGGG					660
	TGGACAACTA					720
	TGCCTGAGGG					
	TCCAGCCGGT				COMMIGICI	780
						813

- (2) INFORMATION FOR SEQ ID NO: 131:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 817 base pairs
    - (B) TYPE: nucleic acid (C) STRANDEDNESS: double
      - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Enterococcus avium
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 131:

CGGAGCTATC TTAGTAGTAT CTGCTGCTGA TGGCCCTATG CCTCAAACTC GTGAACACAT 60
CTTGTTATCT CGTAACGTTG GTGTTCCTTA CATCGTTGTA TTCTTAAACA AAATGGATAT 120
GGTTGACGAT GAAGAATTAC TTGAATTAGT TGAAATGGAA GTTCGTGACT TATTAACTGA 180
ATACGACTTC CCAGGCGACG ACACTCCAGT TATCGCAGGT TCAGCGTTGA AAGCTTTAGA 240
AGGCGACGCT TCATACGAAG AAAAAATCTT AGAATTAATG GCTGCTGTTG ACGAATATAT 300

CCCAACACCA GTTCGTG	TA CTGACAAACC	ATTCATGATG	CCAGTCGAAG	ACGTATTCTC	360	
AATCACTGGT CGTGGTAC	TTG TTGCAACTGG	TCGTGTTGAA	CGTGGACAAG	TTCGCGTTGG	420	
TGACGAAGTT GAAATCG	rag gtatcgctga	CGAAACTGCT	AAAACAACTG	TTACAGGTGT	480	
TGAAATGTTC CGTAAAT	IGT TAGACTACGC	TGAAGCAGGT	GACAACATCG	GTGCTTTGTT	540	
ACGTGGTGTT GCACGTG	AAG ATATCCAACG	TGGACAAGTA	TTGGCTAAAC	CAGCTTCAAT	600	
CACTCCACAT ACAAAAT	TCT CTGCAGAAGT	TTATGTTCTA	ACTAAAGAAG	AAGGTGGACG	660	
TCATACTCCA TTCTTCA	CTA ACTACCGTCC	TCAGTTCTAC	TTCCGTACAA	CTGACGTAAC	720	
TGGTGTAGTT GATCTAC	CAG AAGGTACTGA	AATGGTWATG	CCTGGGGATA	ACGTAACTAT	780	
GGAAGTTGAA TTGATYC	ACC CAATYGCGGT	AGAAGAC			817	
(2) INFORMATION FO	R SEQ ID NO: 1	32:				
(i) SEQUENCE CHARACTERISTICS:						
	(A) LENGTH: 817 base pairs					
	: nucleic acid					
(C) STR	NDEDNESS: doub	)TG				

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Enterococcus faecalis
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 132: CGGAGCTATC TTAGTAGTTT CTGCTGCTGA TGGTCCTATG CCTCAAACAC GTGAACATAT 60 CTTATTATCA CGTAACGTTG GTGTACCATA CATCGTTGTA TTCTTAAACA AAATGGATAT 120 GGTTGATGAC GAAGAATTAT TAGAATTAGT AGAAATGGAA GTTCGTGACT TATTATCAGA 180 ATACGATTTC CCAGGCGATG ATGTTCCAGT TATCGCAGGT TCTGCTTTGA AAGCTTTAGA 240 AGGCGACGAG TCTTATGAAG AAAAAATCTT AGAATTAATG GCTGCAGTTG ACGAATATAT 300 CCCAACTCCA GAACGTGATA CTGACAAACC ATTCATGATG CCAGTCGAAG ACGTATTCTC 360 AATCACTGGA CGTGGTACTG TTGCTACAGG ACGTGTTGAA CGTGGTGAAG TTCGCGTTGG 420 TGACGAAGTT GAAATCGTTG GTATTAAAGA CGAAACATCT AAAACAACYG TTACAGGTGT 480 TGAAATGTTC CGTAAATTAT TAGACTACGC TGAAGCAGGC GACAACMTCG GTGCTTTATT 540 ACGTGGTGTA GCACGTGAAG ATATCGAACG TGGACAAGTA TTAGCTAAAC CAGCTACAAT 600 CACTCCACAC ACAAAATTCA AAGCTGAAGT ATACGTATTA TCAAAAGAAG AAGGCGGACG 660 TCACACTCCA TTCTTCACTA ACTACCGTCC TCAATTCTAC TTCCGTACAA CAGACGTTAC 720

- 124 -

TGGTGTTGTA GAATTGCCAG AAGGTACTGA AATGGTAATG CCTGGTGATA ACGTTGCTAT	780
GGACGTTGAA TTAATTCACC CAATCGCTAT CGAAGAC	817
(2) INFORMATION FOR SEQ ID NO: 133:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 774 base pairs (B) TYPE: nucleic acid (C) STRANDEDMESS: double (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE: (A) ORGANISM: Enterococcus faecium	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 133:	
CGGAGCTATC TTGGTAGTTT CTGCTGCTGA CGGCCCAATG CCTCAAACTC GTGAACACAT	60
CCTATTGTCT CGTCAAGTTG GTGTTCCTTA CATCGTTGTA TTCTTGAACA AAGTAGACAT	120
GGTTGATGAC GAAGAATTAC TAGAATTAGT TGAAATGGAA GTTCGTGACC TATTAACAGA	180
ATACRAATTC CCTGGTGRCG ATGTTCCTGT AGTTGCTGGA TCAGCTTTGA AAGCTCTAGA	240
AGGCGACGCT TCATACGAAG AAAAAATTCT TGAATTAATG GCTGCAGTTG ACGAATACAT	300
CCCAACTCCA GAACGTGACA ACGACAAACC ATTCATGATG CCAGTTGAAG ACGTGTTCTC	360
AATTACTGGA CGTGGTACTG TTGCTACAGG TCGTGTTGAA CGTGGACAAG TTCGCGTTGG	420
TGACGAAGTT GAAGTTGTTG GTATTGCTGA AGAAACTTCA AAAACAACAG TTACTGGTGT	480
GAAATGTTC CGTAAATTGT TAGACYACGC TGAAGCTGGA GACRACATTG GTGCTTTACT	540
ACGTGGTGTT GCACGTGAAG ACATCCAACG TGGACAAGTT TTAGCTAAAC CAGGTACAAT	600
CACACCTORT ACAAAATTOT CTGCAGAAGT ATACGTGTTG ACAAAAGAAG AAGGTGGACG	660
CATACTCCA TTCTTCACTA ACTACCGTCC ACAATTCTAC TTCCGTACAA CTGACGTAAC	720
GGTGTTGTT GAATTACCAG AAGGAACTGA AATGGTCATG CCCGGTGACA ACGT	774
2) INFORMATION FOR SEQ ID NO: 134:	
(i) SEQUENCE CHARACTERISTICS:	

- (A) LENGTH: 809 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:

## (A) ORGANISM: Enterococcus gallinarum

(xi) SE	QUENCE DESC	RIPTION: SE	Q ID NO: 13	34:		
CGGTGCGATC	TTAGTAGTAT	CTGCTGCTGA	CGGTCCTATG	CCTCAAACTC	GTGAACACAT	60
CTTGTTATCA	CGTAACGTTG	GCGTACCATA	CATCGTTGTT	TTCTTGAACA	AAATGGATAT	120
GGTTGAYGAC	GAAGAATTGC	TAGAATTAGT	TGAAATGGAA	GTTCGTGACC	TATTGTCTGA	180
ATATGACTTC	CCAGGCGACG	ATGTTCCTGT	AATCGCCGGT	TCTGCTTTGA	AAGCTCTTGA	240
AGGAGATCCT	TCATACGAAG	AAAAAATCAT	GGAATTGATG	GCTGCAGTTG	ACGAATACGT	300
TCCAACTCCA	GAACGTGATA	CTGACAAACC	ATTCATGATG	CCAGTCGAAG	ACGTATTCTC	360
AATCACTGGA	CGTGGTACTG	TTGCTACAGG	CCGTGTTGAA	CGTGGACAAG	TTCGCGTTGG	420
TGATGAAGTA	GAAATCGTTG	GTATTGCTGA	CGAAACTGCT	AAAACAACTG	TAACAGGTGT	480
TGAAATGTTC	CGTAAATTGT	TAGACTATGC	TGAAGCAGGG	GATAACATTG	GTGCATTGCT	540
ACGTGGGGTT	GCTCGTGAAG	ACATCCAACG	TGGACAAGTA	TTGGCTAAAG	CTGGTACAAT	600
CACACCTCAT	ACAAAATTCA	AAGCTGAAGT	TTATGTTTTG	ACAAAAGAAG	AAGGTGGACG	660
TCACACTCCA	TTCTTCACTA	ACTACCGTCC	TCAGTTCTAC	TTCCGTACAA	CTGACGTAAC	720
TGGTGTTGTT	GAATTACCAG	AAGGAACTGA	AATGGTGATG	CCTGGCGACA	ACGTGACCAT	780
CGACGTTGAA	TTGATRCACC	CAATCGCTC				80

- (2) INFORMATION FOR SEQ ID NO: 135:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 823 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Gardnerella vaginalis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 135:

TGGCGCAATC CTCGTGGTTG CTGCTACCGA CGGTCCAATG GCTCAGACCC GTGAACACGT 60
CTTGCTTGCT AAGCAGGTCG GCGTTCCAAA AATTCTTGTT GCTTTGAACA AGTGCGATAT 120
GGTTGACGAC GAAGAGCTTA TCGATCTCGT TGAAGAAGAG GTCCGTGACC CTCTCGAAGA 180
AAACGGCTTC GATCGCGATT GCCCAGTCYT CCGTACTTCC GCTTACGGCG CTTTGCATGA 240
TGACGCTCCA GACCACGACA AGTGGGTAGA GACCGTCAAG GAACTCATGA AGGCTGTGA 300

CGAGTACATC	CCAACCCCAA	CTCACGATCT	TGACAAGCCA	TTCTTGATGC	CAATCGAAGA	360
TGTGTTCACC	ATCTCCGGTC	GTGGTYCCGT	TGTCACCGGT	CGTGTTGAGC	GTGGTAAGCT	420
CCCAATCAAC	ACCCCAGTTG	AGATCGTTGG	TTTGCGCGAT	ACCCAGACCA	CCACCGTCAC	480
CTCTATCGAG	ACCTTCCACA	AGCAGATGGA	TGAGGCAGAG	GCTGGCGATA	ACACTGGTCT	540
TCTTCTCCGC	GGTATCAACC	GTACCGACGT	TGAGCGTGGT	CAGGTTGTGG	CTGCTCCAGG	600
TTCTGTGACT	CCACACACCA	AGTTCGAAGG	CGAAGTTTAC	GTCTTGACCA	AGGACGAAGG	660
TGGCCGTCAC	TCGCCATTCT	TCTCCAACTA	CCGTCCACAG	TTCTACTTCC	GTACCACCGA	720
TGTTACTGGC	GTTATCACCT	TGCCAGACGG	CATCGAAATG	GTTCAGCCAG	GCGATCACGC	780
AACCTTCACT	GTTGAGTTGA	TCCAGGCTAT	CGCAATGGAA	GAG		823
/2\ T**F0F**						

- (2) INFORMATION FOR SEQ ID NO: 136:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 817 base pairs
    - (B) TYPE: nucleic acid
      (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
     (A) ORGANISM: Listeria innocua
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 136:

CGGAGCTATC TTAGTAGTAT CTGCTGCTGA TGGCCCAATG CCACAAACTC GTGAACATAT 60 CTTACTTTCA CGTCAAGTTG GTGTTCCATA CATCGTTGTA TTCATGAACA AATGTGACAT 120 GGTTGACGAT GAAGAATTAC TAGAATTAGT TGAAATGGAA ATTCGTGATC TATTAACTGA 180 ATATGAATTC CCTGGCGATG ACATTCCTGT AATCAAAGGT TCAGCTCTTA AAGCACTTCA 240 AGGTGAAGCT GACTGGGAAG CTAAAATTGA CGAGTTAATG GAAGCTGTAG ATTCTTACAT 300 TCCAACTCCA GAACGTGATA CTGACAAACC ATTCATGATG CCAGTTGAGG ATGTATTCTC 360 AATCACTGGT CGTGGAACAG TTGCAACTGG ACGTGTTGAA CGTGGACAAG TTAAAGTTGG 420 TGACGAAGTA GAAGTTATCG GTATTGAAGA AGAAAGCAAA AAAGTAGTAG TAACTGGAGT 480 AGAAATGTTC CGTAAATTAC TAGACTACGC TGAAGCTGGC GACAACATTG GCGCACTTCT 540 ACGTGGTGTT GCTCGTGAAG ATATCCAACG TGGTCAAGTA TTAGCTAAAC CAGGTTCGAT 600 TACTCCACAC ACTAACTTCA AAGCTGAAAC TTATGTTTTA ACTAAAGAAG AAGGTGGACG 660 TCACACTCCA TTCTTCAACA ACTACCGCCC ACAATTCTAT TTCCGTACTA CTGACGTAAC 720

780

817

TGGTATTGTT ACACTTCCAG AAGGTACTGA AATGGTAATG CCTGGTGATA ACATTGAGCT

TGCAGTTGAA CTAATTGCAC CAATCGCTAT CGAAGAC

(2) INFORMATION FOR SEQ ID NO: 138:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 817 base pairs

(B) TYPE: nucleic acid

(C) STRANDENNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(2) INFORMATION FOR SEQ ID NO: 137:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 818 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE: (A) ORGANISM: Listeria ivanovii	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 137:	
CGGAGCTATC TTAGTAGTAT CTGCTGCTGA TGGTCCAATG CCACAAACTC GTGAACATAT	60
TCTTACTTC ACGTCAAGTT GGTGTTCCAT ACATCGTTGT ATTCATGAAC AAATGTGACA	120
TGGTTGACGA TGAAGAATTA CTTGAATTAG TTGAAATGGA AATTCGTGAT CTATTAACTG	180
ARTATGAATT CCCTGGCGAC GACATTCCTG TAATCAAAGG TTCAGCTCTT AAAGCACTTC	240
AAGGTGAAGC TGATTGGGAA GCTAAAATTG ACGAGTTAAT GGAAGCTGTA GATTCTTACA	300
TTCCAACTCC AGAACGTGAT ACTGACAAAC CATTCATGAT GCCAGTTGAG GATGTATTCT	360
CAATCACTGG TCGTGGAACA GTTGCAACTG GACGTGTTGA ACGTGGACAA GTTAAAGTTG	420
GTGACGAAGT AGAAGTTATC GGTATTGAAG AAGAAAGCAA AAAAGTAGTA GTAACTGGAG	480
TAGAAATGTT CCGTAAATTA CTAGACTACG CTGAAGCTGG CGACAACATT GGCGCACTTC	540
TACGTGGTGT TGCTCGTGAA GATATCCAAC GTGGTCAAGT ATTAGCTAAA CCAGGTTCGA	600
TTACTCCACA TACTAACTTC AAAGCTGAAA CTTATGTTTT AACTAAAGAA GAAGGTGGAC	660
GTCATACTCC ATTCTTCAAC AACTACCGCC CACAATTCTA TTTCCGTACT ACTGACGTAA	720
CTGGTATTGT TACACTTCCA GAAGGTACTG AAATGGTAAT GCCTGGTGAT AACATTGAGC	78
TTGCAGTTGA ACTAATTGCA CCAATCGCTA TCGAAGAC	81

(vi)		INAL SOURCE		
	(A)	ORGANISM:	Listeria	monocytogenes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 138: CGGAGCTATC TTAGTAGTAT CTGCTGCTGA TGGCCCAATG CCACAAACTC GTGAACATAT 60 CTTACTTCA CGTCAAGTTG GTGTTCCATA CATCGTTGTA TTCATGAACA AATGTGACAT 120 GGTTGACGAT GAAGAATTAC TAGAATTAGT TGAAATGGAA ATTCGTGATC TATTAACTGA 180 ATATGAATTC CCTGGCGATG ACATTCCTGT AATCAAAGGT TCAGCTCTTA AAGCACTTCA 240 AGGTGAAGCT GACTGGGAAG CTAAAATTGA CGAGTTAATG GAAGCTGTAG ATTCTTACAT 300 TCCAACTCCW GAACGTGATA CTGACAAACC ATTCATGATG CCAGTTGAGG ATGTATTCTC 360 AATCACTGGT CGTGGAACAG TTGCAACTGG ACGTGTTGAA CGTGGACAAG TTAAAGTTGG 420 TGACGAAGTA GAAGTTATCG GTATCGAAGA AGAAAGCAAA AAAGTAGTAG TAACTGGAGT 480 AGAAATGTTC CGTAAATTAC TAGACTACGC TGAAGCTGGC GACAACATTG GCGCACTTCT 540 ACGTGGTGTT GCTCGTGAAG ATATCCAACR TGGTCAAGTA TTAGCTAAAC CAGGTTCGAT 600 TACTCCACAC ACTAACTTCA AAGCTGAAAC TTATGTTTTA ACTAAAGAAG AAGGTGGACG 660 TCACACTCCA TTCTTCAACA ACTACCGCCC ACAATTCTAT TTCCGTACTA CTGACGTAAC 720 TGGTATTGTT ACACTTCCAG AAGGTACTGA AATGGTAAYG CCTGGTGATA ACATTGAGCT 780 TGCAGTTGAA CTAATTGCAC CAATCGCTAT CGAAGAC 817

- (2) INFORMATION FOR SEQ ID NO: 139:
  - (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 817 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double

    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Listeria seeligeri
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 139:

CGGAGCTATC TTAGTAGTAT CTGCTGCTGA TGGCCCAATG CCACAAACTC GTGAACATAT 60 CTTACTTCA CGTCAAGTTG GTGTTCCATA CATCGTTGTA TTCATGAACA AATGTGACAT 120 GGTTGACGAT GAAGAATTAC TTGAATTAGT TGAAATGGAA ATTCGTGATC TATTAACTGA 180 ATATGAATTC CCTGGTGATG ACATTCCTGT AATCAAAGGT TCAGCTCTTA AAGCACTTCA 240

AGGTGAAGCT	GACTGGGAAG	CTAAAATTGA	CGAGTTAATG	GAAGCTGTAG	ATTCTTACAT	300
TCCAACTCCA	GAACGTGATA	CTGACAAACC	ATTCATGATG	CCAGTTGAGG	ATGTATTCTC	360
AATCACTGGT	CGTGGAACTG	TTGCAACTGG	ACGTGTTGAA	CGTGGACAAG	TTAAAGTTGG	420
TGACGAAGTA	GAAGTTATCG	GTATTGAAGA	AGAAAGCAAA	AAAGTAATAG	TAACTGGAGT	480
AGAAATGTTC	CGTAAATTAC	TAGACTACGC	TGAAGCTGGC	GACAACATTG	GCGCACTTCT	540
ACGTGGTGTT	GCTCGTGAAG	ATATCCAACG	TGGTCAAGTA	TTAGCTAAAC	CAGGTTCGAT	600
TACTCCACAT	ACTAACTTCA	AAGCTGAAAC	TTATGTTTTA	ACTAAAGAAG	AAGGTGGACG	6 <b>6</b> 0
TCACACTCCA	TTCTTCAACA	ACTACCGCCC	ACAATTCTAT	TTCCGTACTA	CTGACGTAAC	720
TGGTATTGTT	ACACTTCCAG	AAGGTACTGA	AATGGTAATG	CCTGGTGATA	ACATTGAGCT	780
TGCAGTTGAA	CTAATTGCAC	CAATCGCTAT	CGAAGAC			81.

- (2) INFORMATION FOR SEQ ID NO: 140:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 814 base pairs
      - (B) TYPE: nucleic acid
      - (C) STRANDEDNESS: double
      - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Staphylococcus aureus
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 140:

CGGTGGTATC TTAGTAGTAT CTGCTGCTGA CGGTCCAATG CCACAAACTC GTGAACACAT 60 TCTTTTATCA CGTAACGTTG GTGTACCAGC ATTAGTAGTA TTCTTAAACA AAGTTGACAT 120 GGTTGACGAT GAAGAATTAT TAGAATTAGT AGAAATGGAA GTTCGTGACT TATTAAGCGA 180 ATATGACTTC CCAGGTGACG ATGTACCTGT AATCGCTGGT TCAGCATTAR AAGCTTTAGA 240 AGGCGATGCT CAATACGAAG AAAAAATCTT AGAATTARTG GAAGCTGTAG ATACTTACAT 300 TCCAACTCCA GAACGTGATT CTGACAAACC ATTCATGATG CCAGTTGAGG ACGTATTCTC 360 AATCACTGGT CGTGGTACTG TTGCTACAGG CCGTGTTGAA CGTGGTCAAA TCAAAGTTGG 420 TGAAGAAGTT GAAATCATCG GTTTACATGA CACATCTAAA ACAACTGTTA CAGGTGTTGA 480 AATGTTCCGT AAATTATTAG ACTACGCTGA AGCTGGTGAC AACATTGGTG CATTATTACG 540 TGGTGTTGCT CGTGAAGACG TACAACGTGG TCAAGTATTA GCTGCTCCTG GTTCAATTAC 600 ACCACATACT GAATTCAAAG CAGAAGTATA CGTATTATCA AAAGACGAAG GTGGACGTCA 660

CACTCCATTC TTCTCAAACT ATCGTCCACA ATTCTATTTC CGTACTACTG ACGTAACTGG	720
TGTTGTTCAC TTACCAGAAG GTACTGAAAT GGTAATGCCT GGTGATAACG TTGAAATGAC	780
AGTAGAATTA ATCGCTCCAA TCGCGATTGA AGAC	814
(2) INFORMATION FOR SEQ ID NO: 141:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 814 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE: (A) ORGANISM: Staphylococcus epidermidis	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 141:	
CGGCGGTATC TTAGTTGTAT CTGCTGCTGA CGGTCCAATG CCACAAACTC GTGAACACAT	60
CTTATTATCA CGTAACGTTG GTGTACCAGC ATTAGTTGTA TTCTTAAACA AAGTTGACAT	120
GGTAGACGAC GAAGAATTAT TAGAATTAGT TGAAATGGAA GTTCGTGACT TATTAAGCGA	180
ATATGACTTC CCAGGTGACG ATGTACCTGT AATCGCTGGT TCTGCATTAA AAGCATTAGA	240
AGGCGATGCT GAATACGAAC AAAAAATCTT AGACTTAATG CAAGCAGTTG ATGATTACAT	300
TCCAACTCCA GAACGTGATT CTGACAAACC ATTCATGATG CCAGTTGAGG ACGTATTCTC	360
AATCACTGGT CGTGGTACTG TTGCTACAGG CCGTGTTGAA CGTGGTCAAA TCAAAGTWGG	420
TGAAGAAGTT GAAATCATCG GTATGCACGA AACTTCTAAA ACAACTGTTA CTGGTGTAGA	480
AATGTTCCGT AAATTATTAG ACTACGCTGA AGCTGGTGAC AACATCGGTG CTTTATTACG	540
TGGTGTTGCA CGTGAAGACG TACAACGTGG TCAAGTATTA GCTGCTCCTG GTTCTATTAC	600
ACCACACA AAATTCAAAG CTGAAGTATA CGTATTATCT AAAGATGAAG GTGGACGTCA	660
CACTCCATTC TTCACTAACT ATCGCCCACA ATTCTATTTC CRTACTACTG ACGTAACTGG	720
TGTTGTAAAC TTACCAGAAG GTACAGAAAT GGTTATGCCT GGCGACAACG TTGAAATGAC	780
AGTTGAATTA ATCGCTCCAA TCGCTATCGA AGAC	814
(2) INFORMATION FOR SEC ID NO. 142.	

(2) INFORMATION FOR SEQ ID NO: 142:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 817 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 142:

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Staphylococcus saprophyticus

CGGAGCTATC TTAGTAGTAT CTGCTGCTGA TGGCCCCAATG CCACAAACTC GTGAACACAT

TCTTTTATCA CGTRACGTTG GTGYTCCAGC ATTAGTTGTA TTCTTAAACA AAGTTGACAT	120
GGTTGACGAY GAAGAATTAT TAGAATTRGT AGAAATGGAA GTTCGTGRCT TATTAAGCGA	180
ATATGACTTC CCAGGTGACG ATGTACCTGT AATCTCTGGT TCTGCATTAA AAGCTTTAGA	240
AGGCGACGCT GACTATGAGC AAAAAATCTT AGACTTAATG CAAGCTGTTG ATGACTYCAT	300
TCCAACACCA GAACGTGATT CTGACAAACC ATTCATGATG CCAGTTGAGG ACGTATTCTC	360
AATCACTGGT CGTGGTACTG TTGCTACAGG CCGTGTTGAA CGTGGTCAAA TCAAAGTCGG	420
TGAAGAAATC GARATCATCG GTATGCAAGA AGAATCAAGC AAAACAACTG TTACTGGTGT	480
AGAAATGTTC CGTAAATTAT TAGACTACGC TGAAGCTGGT GACAACATTG GTGCATTATT	540
ACGTGGTGTT TCACGTGATG ATGTACAACG TGGTCAAGTT TTAGCTGCTC CTGGTACTAT	600
CACACCACAT ACAAAATTCA AAGCGGATGT TTACGTTTTA TCTAAAGATG AAGGTGGTCG	660
TCATACGCCA TTCTTCACTA ACTACCGCCC ACAATTCTAT TTCCGTACTA CTGACGTAAC	720
TGGTGTTGTT AACTTACCAG AAGGTACTGA AATGGTTATG CCTGGCGATA ACGTTGAAAT	780
GGATGTTGAA TTAATTTCTC CAATCGCTAT TGAAGAC	817
(2) INFORMATION FOR SEQ ID NO: 143:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 817 base pairs  (B) TYPE: nucleic acid  (C) STRANDENNESS: double  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE:  (A) ORGANISM: Staphylococcus simulans	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 143:	
CGGCGGTATC TTAGTAGTAT CTGCTGCAGA TGGTCCAATG CCACAAACTC GTGAACACAT	60
CTTATTATCA CGTAACGTTG GTGTACCAGC TTTAGTTGTA TTCTTAAACA AAGCTGACAT	
CTTATTATCA CGTAACGTTG GTGTACCAGC IIIAGIIGIA IICITAAACA AAGOTO	120

ATACGACTTC	CCTGGTGACG	ATGTACCAGT	TATCGTTGGT	TCTGCATTAA	AAGCTTTAGA	24
AGGCGACCCA	GAATACGAAC	AAAAAATCTT	AGACTTAATG	CAAGCTGTAG	ATGACTACAT	300
CCCAACTCCA	GAACGTGACT	CTGATAAACC	ATTCATGATG	CCAGTTGAGG	ACGTATTCTC	360
AATCACTGGT	CGTGGTACTG	TAGCAACAGG	CCGTGTTGAA	CGTGGTCAAA	TCAAAGTCGG	420
TGAAGAAGTT	GAAATCATCG	GTATCACTGA	AGAAAGCAAG	AAAACAACAG	TTACAGGTGT	480
AGAAATGTTC	CGTAAATTAT	TAGACTACGC	TGAAGCTGGT	GACAACATCG	GTGCTTTATT	540
ACGTGGTGTT	GCACGTGAAG	ACGTACAACG	TGGACAAGTA	TTAGCAGCTC	CTGGCTCTAT	600
TACTCCACAC	ACAAAATTCA	AAGCTGATGT	TTACGTTTTA	TCTAAAGAAG	AAGGTGGACG	660
TCATACTCCA	TTCTTCACTA	ACTACCGCCC	ACAATTCTAC	TTCCGTACTA	CTGACGTAAC	720
TGGCGTTGTT	CACTTACCAG	AAGGTACTGA	AATGGTTATG	CCTGGCGATA	ACGTAGAAAT	780
GACTGTTGAA	TTGATCGCTC	CAATCGCGAT	TGAAGAC			817
(2) THEODIS	mross ===					

- (2) INFORMATION FOR SEQ ID NO: 144:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 817 base pairs
    - (B) TYPE: nucleic acid (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Streptococcus agalactiae (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 144:
- CGGAGCTATC CTTGTAGTTG CTTCAACTGA TGGACCAATG CCACAAACTC GTGAGCACAT 60 CCTTCTTTCA CGTCAAGTTG GTGTTAAACA CCTTATCGTA TTCATGAACA AAGTTGACCT 120 TGTTGATGAT GAAGAATTGC TTGAATTGGT TGAAATGGAA ATTCGTGACC TTCTTTCAGA 180 ATACGACTTC CCAGGTGATG ACCTTCCAGT TATCCAAGGT TCAGCTCTTA AAGCACTTGA 240 AGGCGACGAA AAATACGAAG ACATCATCAT GGAATTGATG AGCACTGTTG ATGAGTACAT 300 TCCAGAACCA GAACGTGATA CTGACAAACC TTTACTTCTT CCAGTTGAAG ATGTATTCTC 360 AATCACTGGA CGTGGTACAG TTGCTTCAGG ACGTATCGAC CGTGGTACTG TTCGTGTCAA 420 CGACGAAGTT GAAATCGTTG GTATTAAAGA AGATATCCAA AAAGCAGTTG TTACTGGTGT 480 TGAAATGTTC CGTAAACAAC TTGACGAAGG TCTTGCAGGG GACAACGTTG GTGTTCTTCT 540 TCGTGGTGTT CAACGTGATG AAATCGAACG TGGTCAAGTT CTTGCTAAAC CAGGTTCAAT 600

CAACCCACAC ACTAAATTTA AAGGTGAAGT TTACATCCTT TCTAAAGAAG AAGGTGGACG	660
TCATACTCCA TTCTTCAACA ACTACCGTCC ACAATTCTAC TTCCGTACAA CTGACGTAAC	720
AGGTTCAATC GAACTTCCAG CAGGAACAGA AATGGTTATG CCTGGTGATA ACGTTACTAT	780
CGAAGTTGAA TTGATTCACC CAATCGCCGT AGAACAA	817
(2) INFORMATION FOR SEQ ID NO: 145:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 817 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE:  (A) ORGANISM: Streptococcus pneumoniae	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 145:	
CGGAGCTATC CTTGTAGTAG CTTCAACTGA CGGACCAATG CCACAAACTC GTGAGCACAT	60
CCTTCTTTCA CGTCAGGTTG GTGTTAAACA CCTTATCGTC TTCATGAACA AAGTTGACTT	120
GGTTGACGAC GAAGAATTGC TTGAATTGGT TGAAATGGAA ATCCGTGACC TATTGTCAGA	180
ATACGACTTC CCAGGTGACG ATCTTCCAGT TATCCAAGGT TCAGCACTTA AAGCTCTTGA	240
AGGTGACTCT AAATACGAAG ACATCGTTAT GGAATTGATG AACACAGTTG ATGAGTATAT	300
CCCAGAACCA GAACGTGACA CTGACAAACC ATTGCTTCTT CCAGTCGAGG ACGTATTCTC	360
AATCACTGGA CGTGGTACAG TTGCTTCAGG ACGTATCGAC CGTGGTATCG TTAAAGTCAA	420
CGACGAAATC GAAATCGTTG GTATCAAAGA AGAAACTCRA AAAGCAGTTG TTACTGGTGT	480
TGAAATGTTC CGTAAACAAC TTGACGAAGG TCTTGCTGGA GATAACGTAG GTGTCCTTCT	540
TCGTGGTGTT CAACGTGATG AAATCGAACG TGGACAAGTT ATCGCTAAAC CAGGTTCAAT	600
CAACCCACAC ACTARATTCA AAGGTGAAGT CTACATCCTT ACTARAGAAG AAGGTGGACG	660
TCACACTCCA TTCTTCAACA ACTACCGTCC ACAATTCTAC TTCCGTACTA CTGACGTTAC	720
AGGTTCAATC GAACTTCCAG CAGGTACTGA AATGGTAATG CCTGGTGATA ACGTGACAAT	780
CGACGTTGAG TTGATTCACC CAATCGCCGT AGAACAA	817
(2) INFORMATION FOR SEQ ID NO: 146:	

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 817 base pairs
 (B) TYPE: nucleic acid

(C) STRANDEDNESS: double (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
<ul><li>(vi) ORIGINAL SOURCE:</li><li>(A) ORGANISM: Streptococcus salivarius</li></ul>	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 146:	
CGGTGCGATC CTTGTAGTAG CATCTACTGA CGGACCAATG CCACAAACTC GTGAGCACAT	
	6
CCTTCTTTCA CGTCAGGTTG GTGTTAAACA CCTTATCGTC TTCATGAACA AAGTTGACTT	12
GGTTGACGAT GAAGAATTGC TTGAATTGGT TGAAATGGAA ATCCGTGACC TTCTTTCAGA	18
ATACGATTTC CCAGGTGATG ACATTCCAGT TATCCAAGGT TCAGCTCTTA AAGCTCTTGA	24
AGGTGATTCT AAATACGAAG ACATCATCAT GGACTTGATG AACACTGTTG ACGAATACAT	30
CCCAGAACCA GAACGTGACA CTGACAAACC ATTGTTGCTT CCAGTCGAAG ACGTATTCTC	36
AATCACTGGT CGTGGTACTG TTGCTTCAGG ACGTATCGAC CGTGGTGTTG TTCGTGTCAA	420
TGACGAAGTT GAAATCGTTG GTCTTAAAGA AGACATCCAA AAAGCAGTTG TTACTGGTGT	480
TGAAATGTTC CGTAAACAAC TTGACGRAGG TATTGCCGGA GATAACGTCG GTGTTCTTCT	540
TCGTGGTATC CAACGTGATG AAATCGAACG TGGTCAAGTA TTGGCTGCAC CTGGTTCAAT	
CAACCCACAC ACTAAATTCA AAGGTGAAGT TTACATCCTT TCTAAAGAAG AAGGTGGACG	600
TCACACTCCA TTCTTCAACA ACTACCGTCC ACAGTTCTAC TTCCGTACAA CTGACGTAAC	660
	720
AGGTTCAATC GAACTTCCTG CAGGTACTGA AATGGTTATG CCTGGTGATA ACGTGACTAT	780
CGACGTTGAG TTGATCCACC CAATCGCCGT TGAACAA	817
(2) INFORMATION FOR SEQ ID NO: 147:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 997 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE:  (A) ORGANISM: Agrobacterium tumefaciens	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 147:	
AACATGATCA CCGGTGCTGC CGAGATGGAC GGCGCGATCC TGGTTTGCTC GGCTGCCGAC	
GGCCCGATGC CACAGACCCG CGAGCACATC CTGCTTGCCC GTCAGGTGGG CGTTCCGGCC	60
TOTAL STATE OF THE	120

ATCGTCGTGT	TCCTCAACAA	GGTCGACCAG	GTTGACGACG	CCGAGCTTCT	CGAGCTCGTC	180
GAGCTTGAAG	TTCGCGAACT	TCTGTCGTCC	TACGACTTCC	CGGGCGACGA	TATCCCGATC	240
ATCAAGGGTT	CGGCACTTGC	TGCTCTTGAA	GATTCTGACA	AGAAGATCGG	TGAAGACGCG	300
ATCCGCGAGC	TGATGGCTGC	TGTCGACGCC	TACATCCCGA	CGCCTGAGCG	TCCGATCGAC	360
CAGCCGTTCC	TGATGCCGAT	CGAAGACGTG	TTCTCGATCT	CGGGTCGTGG	TACGGTTGTG	420
ACGGGTCGCG	TTGAGCGCGG	TATCGTCAAG	GTTGGTGAAG	AAGTCGAAAT	CGTCGGCATC	480
CGTCCGACCT	CGAAGACGAC	TGTTACCGGC	GTTGAAATGT	TCCGCAAGCT	GCTCGACCAG	540
GGCCAGGCCG	GCGACAACAT	CGGTGCACTC	GTTCGCGGCG	TTACCCGTGA	CGGCGTCGAG	600
CGTGGTCAGA	TCCTGTGCAA	GCCGGGTTCG	GTCAAGCCGC	ACAAGAAGTT	CATGGCAGAA	660
GCCTACATCO	TGACGAAGGA	AGAAGGCGGC	CGTCATACGC	CGTTCTTCAC	GAACTACCGT	720
CCGCAGTTCT	ACTTCCGTAC	GACTGACGTT	ACCGGTATCG	TTTCGCTTCC	TGAAGGCACG	78
GAAATGGTT	TGCCTGGCGA	CAACGTCACT	GTTGAAGTCG	AGCTGATCGT	TCCGATCGCG	84
ATGGAAGAA	A AGCTGCGCT	CGCTATCCGC	GAAGGCGGCC	GTACCGTCGG	CGCCGGC	89

- (2) INFORMATION FOR SEQ ID NO: 148:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 885 base pairs
    - (B) TYPE: nucleic acid(C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - .--,
  - (vi) ORIGINAL SOURCE:
     (A) ORGANISM: Bacillus subtilis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 148:

ATGATCACTG GTGCTGCGCA AATGGACGGA GCTATCCTTG TAGTATCTGC TGCTGATGGC 60 CCAATGCCAC AAACTCGTGA GCACATCCTT CTTTCTAAAA ACGTTGGTGT ACCATACATC 120 GTTGTATTCT TAAACAAATG CGACATGGTA GACGACGAAG AGCTTCTTGA ACTAGTTGAA 180 ATGGAAGTTC GCGATCTTCT TAGCGAATAC GACTTCCCTG GTGATGATGT ACCAGTTGTT 240 AAAGGTTCTG CTCTTAAAGC TCTTGAAGGA GACGCTGAGT GGGAAGCTAA AATCTTCGAA 300 CTTATGGATG CGGTTGATGA GTACATCCCA ACTCCAGAAC GCGACACTGA AAAACCATTC 360 ATGATGCCAG TTGAGGACGT ATTCTCAATC ACTGGTCGTG GTACAGTTGC TACTGGCCGT 420 GTAGAACGCG GACAAGTTAA AGTCGGTGAC GAAGTTGAAA TCATCGGTCT TCAAGAAGAG 480

AA	CAAGAAAA	CAACTGTTAC	AGGTGTTGAA	ATGTTCCGTA	AGCTTCTTGA	TTACGCTGAA	540
GC	TGGTGACA	ACATTGGTGC	CCTTCTTCGC	GGTGTATCTC	GTGAAGAAAT	CCAACGTGGT	600
CA	AGTACTTG	CTAAACCAGG	TACAATCACT	CCACACAGCA	AATTCAAAGC	TGAAGTTTAC	660
GT	TCTTTCTA	AAGAAGAGGG	TGGACGTCAT	ACTCCATTCT	TCTCTAACTA	CCGTCCTCAG	720
тт	CTACTTCC	GTACAACTGA	CGTAACTGGT	ATCATCCATC	TTCCAGAAGG	CGTAGAAATG	780
GT	TATGCCTG	GAGATAACAC	TGAAATGAAC	GTTGAACTTA	TTTCTACAAT	CGCTATCGAA	840
GA.	AGGAACTC	GTTTCTCTAT	TCGTGAAGGC	GGACGTACTG	TTGGT		885
10							

- (2) INFORMATION FOR SEQ ID NO: 149:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 882 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
      (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Bacteroides fragilis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 149:

ATGGTTACTG GTGCTGCTCA GATGGACGGT GCTATCATTG TAGTTGCTGC TACTGATGGT 60 CCGATGCCTC AGACTCGTGA GCACATCCTT TTGGCTCGTC AGGTAAACGT TCCGAAGCTG 120 GTTGTATTCA TGAACAAGTG CGATATGGTT GAAGATGCTG AGATGTTGGA ACTTGTTGAA 180 ATGGAAATGA GAGAATTGCT TTCATTCTAT GATTTCGACG GTGACAATAC TCCGATCATT 240 CAGGGTTCTG CTCTTGGTGC ATTGAACGGC GTAGAAAAAT GGGAAGACAA AGTAATGGAA 300 CTGATGGAAG CTGTTGATAC TTGGATTCCA CTGCCTCCGC GCGATGTTGA TAAACCTTTC 360 TTGATGCCGG TAGAAGACGT GTTCTCTATC ACAGGTCGTG GTACTGTAGC TACAGGTCGT 420 ATCGAAACTG GTGTTATCCA TGTAGGTGAT GAAATCGAAA TCCTCGGTTT GGGTGAAGAT 480 AAGAAATCAG TTGTAACAGG TGTTGAAATG TTCCGCAAAC TTCTGGATCA GGGTGAAGCT 540 GGTGACAACG TAGGTCTGTT GCTTCGTGGT GTTGACAAGA ACGAAATCAA ACGTGGTATG 600 GTTCTTTGTA AACCGGGTCA GATTAAACCT CACTCTAAAT TCAAAGCAGA GGTTTATATC 660 CTGAAGAAG AAGAAGGTGG TCGTCACACT CCATTCCATA ACAAATATCG TCCTCAGTTC 720 TACCTGCGTA CTATGGACTG TACAGGTGAA ATCACTCTTC CGGAAGGAAC TGAAATGGTA 780 ATGCCGGGTG ATAACGTAAC TATCACTGTA GAGTTGATCT ATCCGGTTGC ACTGAACATC 840

GGTCTTCGTT TCGCTATCCG CGAAGGTGGA CGTACAGTAG GT	882
(2) INFORMATION FOR SEQ ID NO: 150:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 888 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDNESS: double  (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE:  (A) ORGANISM: Borrelia burgdorferi	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 150:	
AATATGATTA CAGGAGCAGC TCAAATGGAT GCAGCGATAC TTTTAGTTGC TGCTGATAGT	60
GGTGCTGAGC CTCAAACAAA AGAGCATTTG CTTCTTGCTC AAAGAATGGG AATAAAGAAA	120
ATAATAGTTT TTTTAAATAA ATTGGACTTA GCAGATCCTG AACTTGTTGA GCTTGTTGAA	180
GTTGAAGTTT TAGAACTTGT TGAAAAATAT GGCTTTTCAG CTGATACTCC AATAATCAAA	240
GGTTCAGCTT TTGGGGCTAT GTCAAATCCA GAAGATCCTG AATCTACAAA ATGCGTTAAA	300
GAACTTCTTG AATCTATGGA TAATTATTTT GATCTTCCAG AAAGAGATAT TGACAAGCCA	360
TTTTTGCTTG CTGTTGAAGA TGTATTTCT ATTTCAGGAA GAGGCACTGT TGCTACTGGG	420
CGTATTGAAA GAGGTATTAT TAAAGTTGGT CAAGAAGTTG AAATAGTTGG AATTAAAGAA	480
ACCAGAAAAA CTACTGTTAC TGGTGTTGAA ATGTTCCAGA AAATTCTTGA GCAAGGTCAA	540
GCAGGGGATA ATGTTGGTCT TCTTTTGAGA GGCGTTGATA AAAAAGACAT TGAGAGGGGG	600
CAAGTTTTGT CAGCTCCAGG TACAATTACT CCACACAAGA AATTTAAAGC TTCAATTTAT	660
TGTTTGACTA AAGAAGAAGG CGGTAGGCAC AAGCCATTTT TCCCAGGGTA TAGACCACAG	720
TTCTTTTTTA GAACAACCGA TGTTACTGGA GTTGTTGCTT TAGAGGGCAA AGAAATGGTT	780
ATGCCTGGTG ATAATGTTGA TATTATTGTT GAGCTGATCT CTTCAATAGC TATGGATAAG	840
AATGTAGAAT TTGCTGTTCG AGAAGGTGGA AGAACCGTTG CTTCAGGA	88
(2) INFORMATION FOR SEQ ID NO: 151:	
(i) SEQUENCE CHARACTERISTICS:  (A) LENGTH: 894 base pairs  (B) TYPE: nucleic acid  (C) STRANDEDMESS: double  (D) TOPOLOGY: linear	

(ii) MOLECULE TYPE: DNA (genomic)

- 138 -

		SOURCE		
(A)	ORGZ	NISM:	Brevibacterium	linens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 151:

	CCGGTGCCGC					60
	CCCAGACCCG					120
ATCGTCGTGG	CTCTGAACAA	GTCCGACATG	GTCGATGACG	AGGAGCTCCT	CGAGCTCGTC	180
GAATTCGAGG	TCCGCGACCT	GCTCTCGAGC	CAGGACTTCG	ACGGAGACAA	CGCTCCGGTC	240
ATTCCGGTGT	CCGCTCTCAA	GGCGCTGGAA	GGCGACGAGA	AGTGGGTCAA	GAGCGTTCAG	300
GATCTCATGG	CTGCCGTCGA	TGACAACGTT	CCGGAGCCGG	AGCGCGATGT	CGACAAGCCG	360
TTCCTCATGC	CCGTCGAGGA	CGTCTTCACG	ATCACCGGTC	GTGGAACCGT	CGTCACCGGT	420
CGTGTCGAGC	GCGGCGTGCT	CCTGCCTAAC	GACGAAATCG	AAATCGTCGG	CATCAAGGAG	480
AAGTCGTCCA	AGACGACTGT	CACCGCTATC	GAGATGTTCC	GCAAGACCCT	GCCGGATGCC	540
CGTGCAGGTG	AGAACGTCGG	TCTGCTCCTC	CGCGGCACCA	AGCGCGAGGA	TGTTGAGCGC	600
GGTCAGGTCA	TCGTGAAGCC	GGGTTCGATC	ACCCCGCACA	CCAAGTTCGA	GGCTCAGGTC	660
TACATCCTGA	GCAAGGACGA	GGGCGGACGT	CACAACCCGT	TCTACTCGAA	CTACCGTCCG	720
CAGTTCTACT	TCCGGACCAC	GGACGTCACC	GGTGTCATCA	CGCTGCCCGA	GGGCACCGAG	780
ATGGTCATGC	CCGGCGACAA	CACCGATATG	TCGGTCGAGC	TCATCCAGCC	GATCGCTATG	840
GAGGACCGCC	TCCGCTTCGC	AATCCGCGAA	GGTGGCCGCA	CCGTCGGCGC	CGGT	894

- (2) INFORMATION FOR SEQ ID NO: 152:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 888 base pairs (B) TYPE: nucleic acid

    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:

(A) ORGANISM: Burkholderia cepacia

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 152:

ATGATCACGG GCGCAGCGCA GATGGACGGC GCGATCCTGG TTTGCTCGGC AGCAGACGGC 60 CCGATGCCGC AAACGCGTGA GCACATCCTG CTGGCGCGTC AGGTTGGTGT TCCGTACATC 120 ATCGTGTTCC TGAACAAGTG CGACAGTGTG GACGACGCTG AACTGCTCGA GCTGGTCGAG 180

the state of the s

ATGGAAGTTC	GCGAACTCCT	GTCGAAGTAC	GACTTCCCGG	GCGACGACAC	GCCGATCGTG	240
AAGGGTTCGG	CCAAGCTGGC	GCTGGAAGGC	GACACGGGCG	AGCTGGGCGA	AGTGGCGATC	300
ATGAGCCTGG	CAGACGCGCT	GGACACGTAC	ATCCCGACGC	CGGAGCGTGC	AGTTGACGGC	360
GCGTTCCTGA	TGCCGGTGGA	AGACGTGTTC	TCGATCTCGG	GCCGTGGTAC	GGTGGTGACG	420
GGTCGTGTCG	AGCGCGGCAT	CGTGAAGGTC	GGCGAAGAAA	TCGAAATCGT	CGGTATCAAG	480
CCGACGGTGA	AGACGACCTG	CACGGGCGTT	GAAATGTTCC	GCAAGCTGCT	GGACCAAGGT	540
CAGGCAGGCG	ACAACGTCGG	TATCCTGCTG	CGCGGCACGA	AGCGTGAAGA	CGTGGAGCGT	600
GGCCAGGTTC	TGGCGAAGCC	GGGTTCGATC	ACGCCGCACA	CGCACTTCAC	GGCTGAAGTG	660
TACGTGCTGA	GCAAGGACGA	AGGCGGCCGT	CACACGCCGT	TCTTCAACAA	CTACCGTCCG	72
CAGTTCTACT	TCCGTACGAC	GGACGTGACG	GGCTCGATCG	AGCTGCCGAA	GGACAAGGAA	78
ATGGTGATGC	CGGGCGACAA	CGTGTCGATC	ACGGTGAAGC	TGATTGCTCC	GATCGCGATG	84
GAAGAAGGTO	TGCGCTTCGC	AATCCGTGAA	GGCGGCCGTA	CGGTCGGC		88

- (2) INFORMATION FOR SEQ ID NO: 153:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 891 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Chlamydia trachomatis
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 153:

AACATGATCA CCGGTGCGGC TCAAATGGAC GGGGCTATTC TAGTAGTTTC TGCAACAGAC 60 GGAGCTATGC CTCAAACTAA AGAGCATATT CTTTTGGCAA GACAAGTTGG GGTTCCTTAC 120 ATCGTTGTTT TTCTCAATAA AATTGACATG ATTTCCGAAG AAGACGCTGA ATTGGTCGAC 180 TTGGTTGAGA TGGAGTTGGC TGAGCTTCTT GAAGAGAAAG GATACAAAGG GTGTCCAATC 240 ATCAGAGGTT CTGCTCTGAA AGCTTTGGAA GGAGATGCTG CATACATAGA GAAAGTTCGA 300 GAGCTAATGC AAGCCGTCGA TGATAATATC CCTACTCCAG AAAGAGAAAT TGACAAGCCT 360 TTCTTAATGC CTATTGAGGA CGTGTTCTCT ATCTCCGGAC GAGGAACTGT AGTAACTGGA 420 CGTATTGAGC GTGGAATTGT TAAAGTTTCC GATAAAGTTC AGTTGGTCGG TCTTAGAGAT 480 ACTAAAGAAA CGATTGTTAC TGGGGTTGAA ATGTTCAGAA AAGAACTCCC AGAAGGTCGT 540

GCAGGAGAGA ACGTTGGATT GCTCCTCAGA GGTATTGGTA AGAACGATGT GGAAAGAGGA	60
ATGGTTGTTT GCTTGCCAAA CAGTGTTAAA CCTCATACAC AGTTTAAGTG TGCTGTTTAC	660
GTTCTGCAAA AAGAAGAAGG TGGACGACAT AAGCCTTTCT TCACAGGATA TAGACCTCAA	720
TTCTTCTTCC GTACAACAGA CGTTACAGGT GTGGTAACTC TGCCTGAGGG AGTTGAGATG	780
GTCATGCCTG GGGATAACGT TGAGTTTGAA GTGCAATTGA TTAGCCCTGT GGCTTTAGAA	840
GAAGGTATGA GATTTGCGAT TCGTGAAGGT GGTCGTACAA TCGGTGCTGG A	891
(2) INFORMATION FOR SEC ID NO. 15.	

- (2) INFORMATION FOR SEQ ID NO: 154:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 891 base pairs (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Escherichia coli (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 154:

AACATGATCA CCGGTGCTGC GCAGATGGAC GGCGCGATCC TGGTAGTTGC TGCGACTGAC 60 GGCCCGATGC CGCAGACTCG TGAGCACATC CTGCTGGGTC GTCAGGTAGG CGTTCCGTAC 120 ATCATCGTGT TCCTGAACAA ATGCGACATG GTTGATGACG AAGAGCTGCT GGAACTGGTT 180 GAAATGGAAG TTCGTGAACT TCTGTCTCAG TACGACTTCC CGGGCGACGA CACTCCGATC 240 GTTCGTGGTT CTGCTCTGAA AGCGCTGGAA GGCGACGCAG AGTGGGAAGC GAAAATCCTG 300 GAACTGGCTG GCTTCCTGGA TTCTTACATT CCGGAACCAG AGCGTGCGAT TGACAAGCCG 360 TTCCTGCTGC CGATCGAAGA CGTATTCTCC ATCTCCGGTC GTGGTACCGT TGTTACCGGT 420 CGTGTAGAAC GCGGTATCAT CAAAGTTGGT GAAGAAGTTG AAATCGTTGG TATCAAAGAG 480 ACTCAGAAGT CTACCTGTAC TGGCGTTGAA ATGTTCCGCA AACTGCTGGA CGAAGGCCGT 540 GCTGGTGAGA ACGTAGGTGT TCTGCTGCGT GGTATCAAAC GTGAAGAAAT CGAACGTGGT 600 CAGGTACTGG CTAAGCCGGG CACCATCAAG CCGCACACCA AGTTCGAATC TGAAGTGTAC 660 ATTCTGTCCA AAGATGAAGG CGGCCGTCAT ACTCCGTTCT TCAAAGGCTA CCGTCCGCAG 720 TTCTACTTCC GTACTACTGA CGTGACTGGT ACCATCGAAC TGCCGGAAGG CGTAGAGATG 780 GTAATGCCGG GCGACAACAT CAAAATGGTT GTTACCCTGA TCCACCCGAT CGCGATGGAC 840 GACGGTCTGC GTTTCGCAAT CCGTGAAGGC GGCCGTACCG TTGGCGCGGG C 891

(2)	INFORMATION	FOR	SEQ	ID	NO:	155:
-----	-------------	-----	-----	----	-----	------

(i)	SEQUENCE	CHARACTERISTICS	:

- (A) LENGTH: 891 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (C) STRANDEDNESS: dour (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Fibrobacter succinogenes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 155:

AACATGGTGA	CTGGTGCTGC	TCAGATGGAC	GGCGCTATCC	TCGTTGTTGC	CGCTACTGAC	60
GGTCCGATGC	CGCAGACTCG	CGAACACATC	CTTCTCGCTC	ACCAGGTTGG	CGTGCCGAAG	120
ATCGTCGTGT	TCATGAACAA	GTGCGACATG	GTTGACGATG	CTGAAATTCT	CGACCTCGTC	180
GAAATGGAAG	TTCGCGAACT	CCTCTCCAAG	TATGACTTCG	ACGGTGACAA	CACCCCGATC	240
ATCCGTGGTT	CCGCTCTCAA	GGCCCTCGAA	GGCGATCCGG	AATACCAGGA	CAAGGTCATG	300
GAACTCATGA	ACGCTTGCGA	CGAATACATC	CCGCTCCCGC	AGCGCGATAC	CGACAAGCCG	360
	CGATCGAAGA					420
					TCTCGGTGAA	480
	ACGTCATCAC					540
	ACGTTGGTCT		mak)			600
	CAGCTCCGAA					660
					CCGTCCGCAG	72
					TGTCGAAATG	78
					r CGCTATGGAA	84
	C GCTTCGCTA					89
MMGCMGCIC						

## (2) INFORMATION FOR SEQ ID NO: 156:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 894 base pairs
  - (B) TYPE: nucleic acid(C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:

## - 142 -

(A)	ORGANISM:	Flavobacterium	ferrugineur
-----	-----------	----------------	-------------

(Xi)	SEQUENCE	DESCRIPTION:	SEQ	ID	NO:	156:
------	----------	--------------	-----	----	-----	------

AACATGATCA	CCGGTGCTGC	CCAGATGGAC	GGTGCTATCT	TAGTTGTGG	TGCATCAGAC	60
GGTCCTATGC	CTCAAACAAA	AGAACACATC	CTGCTTGCTG	CCCAGGTAGG	TGTACCTAAA	120
ATGGTTGTGT	TTCTGAATAA	AGTTGACCTC	GTTGACGACG	AAGAGCTCCT	GGAGCTGGTT	180
GAGATCGAGG	TTCGCGAAGA	ACTGACTAAA	CGCGGTTTCG	ACGGCGACAA	CACTCCAATC	240
ATCAAAGGTT	CCGCTACAGG	CGCCCTCGCT	ĢĢTGAAGAAA	AGTGGGTTAA	AGAAATTGAA	300
AACCTGATGG	ACGCTGTTGA	CAGCTACATC	CCACTGCCTC	CTCGTCCGGT	TGATCTGCCG	360
TTCCTGATGA	GCGTAGAGGA	CGTATTCTCT	ATCACTGGTC	GTGGTACTGT	TGCTACCGGT	420
CGTATCGAGC	GTGGCCGTAT	CAAAGTTGGT	GAGCCTGTTG	AGATCGTAGG	TCTGCAGGAG	480
TCTCCCCTGA	ACTCTACCGT	TACAGGTGTT	GAGATGTTCC	GCAAACTCCT	CGACGAAGGT	540
GAAGCTGGTG	ATAACGCCGG	TCTCCTCCTC	CGTGGTGTTG	алалалсаса	GATCCGTCGC	600
GGTATGGTAA	TCGTTAAACC	CGGTTCCATC	ACTCCGCACA	CGGACTTCAA	AGGCGAAGTT	660
TACGTACTGA	GCAAAGACGA	AGGTGGCCGT	CACACTCCAT	TCTTCAACAA	ATACCGTCCT	720
CAATTCTACT	TCCGTACAAC	TGACGTTACA	GGTGAAGTAG	AACTGAACGC	AGGAACAGAA	780
ATGGTTATGC	CTGGTGATAA	CACCAACCTG	ACCGTTAAAC	TGATCCAACC	GATCGCTATG	840
GAAAAAGGTC	TGAAATTCGC	GATCCGCGAA	GGTGGCCGTA	CCGTAGGTGC	AGGA	894

- (2) INFORMATION FOR SEQ ID NO: 157:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 891 base pairs (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)

  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Haemophilus influenzae
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 157:

AATATGATTA CTGGTGCGGC ACAAATGGAT GGTGCTATTT TAGTAGTAGC AGCAACAGAT 60 GGTCCTATGC CACAAACTCG TGAACACATC TTATTAGGTC GCCAAGTAGG TGTTCCATAC 120 ATCATCGTAT TCTTAAACAA ATGCGACATG GTAGATGACG AAGAGTTATT AGAATTAGTC 180 GAAATGGAAG TTCGTGAACT TCTATCTCAA TATGACTTCC CAGGTGACGA TACACCAATC 240

a was to the same same same as a s

GTACGTGGTT	CAGCATTACA	AGCGTTAAAC	GGCGTAGCAG	AATGGGAAGA	AAAAATCCTT	300
GAGTTAGCAA	ACCACTTAGA	TACTTACATC	CCAGAACCAG	AACGTGCGAT	TGACCAACCG	360
TTCCTTCTTC	CAATCGAAGA	TGTGTTCTCA	ATCTCAGGTC	GTGGTACTGT	AGTAACAGGT	420
CGTGTAGAAC	GAGGTATTAT	CCGTACAGGT	GATGAAGTAG	AAATCGTCGG	TATCAAAGAT	480
ACAGCGAAAA	CTACTGTAAC	GGGTGTTGAA	ATGTTCCGTA	AATTACTTGA	CGAAGGTCGT	54
GCAGGTGAAA	ACATCGGTGC	ATTATTACGT	GGTACCAAAC	GTGAAGAAAT	CGAACGTGGT	60
CAAGTATTAG	CGAAACCAGG	TTCAATCACA	CCACACACTG	ACTTCGAATC	AGAAGTGTAC	66
GTATTATCAA	AAGATGAAGG	TGGTCGTCAT	ACTCCATTCT	TCAAAGGTTA	CCGTCCACAA	72
TTCTATTTCC	GTACAACAGA	CGTGACTGGT	ACAATCGAAT	TACCAGAAGG	CGTGGAAATG	78
GTAATGCCAG	GCGATAACAT	CAAGATGACA	GTAAGCTTAA	TCCACCCAAT	TGCGATGGAT	84
CAAGGTTTAC	GTTTCGCAAT	CCGTGAAGGT	GGCCGTACAG	TAGGTGCAGG	С	89

- (2) INFORMATION FOR SEQ ID NO: 158:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 906 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:

    (A) ORGANISM: Helicobacter pylori
    - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 158:

AACATGATCA CCGGTGCGGC GCAAATGGAC GGAGCGATTT TGGTTGTTTC TGCAGCTGAT 60 GGCCCTATGC CTCAAACTAG GGAGCATATC TTATTGTCTC GTCAAGTAGG CGTGCCTCAC 120 ATCGTTGTTT TCTTAAACAA ACAAGACATG GTAGATGACC AAGAATTGTT AGAACTTGTA 180 GAAATGGAAG TGCGCGAATT GTTGAGCGCG TATGAATTTC CTGGCGATGA CACTCCTATC 240 GTAGCGGGTT CAGCTTTAAG AGCTTTAGAA GAAGCAAAGG CTGGTAATGT GGGTGAATGG 300 GGTGAAAAAG TGCTTAAACT TATGGCTGAA GTGGATGCCT ATATCCCTAC TCCAGAAAGA 360 GACACTGAAA AAACTTTCTT GATGCCGGTT GAAGATGTGT TCTCTATTGC GGGTAGAGGG 420 ACTGTGGTTA CAGGTAGGAT TGAAAGAGGC GTGGTGAAAG TAGGCGATGA AGTGGAAATC 480 GTTGGTATCA GACCTACACA AAAAACGACT GTAACCGGTG TAGAAATGTT TAGGAAAGAG 540 TTGGAAAAAG GTGAAGCCGG CGATAATGTG GGCGTGCTTT TGAGAGGAAC TAAAAAAGAA 600

GAAGTGGAAC	GCGGTATGGT	TCTATGCAAA	CCAGGTTCTA	TCACTCCGCA	CAAGAAATTT	660
GAGGGAGAAA	TTTATGTCCT	TTCTAAAGAA	GAAGGCGGGA	GACACACTCC	ATTCTTCACC	720
AATTACCGCC	CGCAATTCTA	TGTGCGCACA	ACTGATGTGA	CTGGCTCTAT	CACCCTTCCT	780
GAAGGCGTAG	AAATGGTTAT	GCCTGGCGAT	AATGTGAAAA	TCACTGTAGA	GTTGATTAGC	840
CCTGTTGCGT	TAGAGTTGGG	AACTAAATTT	GCGATTCGTG	AAGGCGGTAG	GACCGTTGGT	900
GCTGGT						
						906

- (2) INFORMATION FOR SEQ ID NO: 159:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 891 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Micrococcus luteus
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 159:

AACATGATCA CCGGCGCGC TCAGATGGAC GGCGCGATCC TCGTGGTCGC CGCTACCGAC 60 GGCCCGATGG CCCAGACCCG TGAGCACGTG CTCCTGGCCC GCCAGGTCGG CGTGCCGGCC 120 CTGCTCGTGG CCCTGAACAA GTCGGACATG GTGGAGGACG AGGAGCTCCT CGAGCGTGTC 180 GAGATGGAGG TCCGGCAGCT GCTGTCCTCC AGGAGCTTCG ACGTCGACGA GGCCCCGGTC 240 ATCCGCACCT CCGCTCTGAA GGCCCTCGAG GGCGACCCCC AGTGGGTCAA GTCCGTCGAG 300 GACCTCATGG ATGCCGTGGA CGAGTACATC CCGGACCCGG TGCGCGACAA GGACAAGCCG 360 TTCCTGATGC CGATCGAGGA CGTCTTCACG ATCACCGGCC GTGGCACCGT GGTGACCGGT 420 CGCGCCGAGC GCGGCACCCT GAAGATCAAC TCCGAGGTCG AGATCGTCGG CATCCGCGAC 480 GTGCAGAAGA CCACTGTCAC CGGCATCGAG ATGTTCCACA AGCAGCTCGA CGAGGCCTGG 540 GCCGGCGAGA ACTGCGGTCT GCTCGTGCGC GGTCTGAAGC GCGACGACGT CGAGCGCGGC 600 CAGGTGCTGG TGGAGCCGGG CTCCATCACC CCGCACACCA ACTTCGAGGC GAACGTCTAC 660 ATCCTGTCCA AGGACGAGGG TGGGCGTCAC ACCCCGTTCT ACTCGAACTA CCGCGCGCAG 720 TTCTACTTCC GCACCACCGA CGTCACCGGC GTCATCACGC TGCCCGAGGG CACCGAGATG 780 GTCATGCCCG GCGACACCAC CGAGATGTCG GTCGAGCTCA TCCAGCCGAT CGCCATGGAG 840 GAGGGCCTCG GCTTCGCCAT CCGCGAGGGT GGCCGCACCG TGGGCTCCGG C 891

(2) INFORMATION FOR SEQ ID NO: 16	121	THEORMATTON	FOR	SEO	ID	NO:	160
-----------------------------------	-----	-------------	-----	-----	----	-----	-----

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 891 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Mycobacterium tuberculosis
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 160:

AACATGATCA	CCGGCGCCGC	GCAGATGGAC	GGTGCGATCC	TGGTGGTCGC	CGCCACCGAC	60
GGCCCGATGC	CCCAGACCCG	CGAGCACGTT	CTGCTGGCGC	GTCAAGTGGG	TGTGCCCTAC	120
ATCCTGGTAG	CGCTGAACAA	GGCCGACGCA	GTGGACGACG	AGGAGCTGCT	CGAACTCGTC	180
GAGATGGAGG	TCCGCGAGCT	GCTGGCTGCC	CAGGAATTCG	ACGAGGACGC	CCCGGTTGTG	240
CGGGTCTCGG	CGCTCAAGGC	GCTCGAGGGT	GACGCGAAGT	GGGTTGCCTC	TGTCGAGGAA	300
CTGATGAACG	CGGTCGACGA	GTCGATTCCG	GACCCGGTCC	GCGAGACCGA	CAAGCCGTTC	36
CTGATGCCGG	TCGAGGACGT	CTTCACCATT	ACCGGCCGCG	GAACCGTGGT	CACCGGACGT	420
GTGGAGCGCG	GCGTGATCAA	CGTGAACGAG	GAAGTTGAGA	TCGTCGGCAT	TCGCCCATCG	48
ACCACCAAGA	CCACCGTCAC	CGGTGTGGAG	ATGTTCCGCA	AGCTGCTCGA	CCAGGGCCAG	54
GCGGGCGACA	ACGTTGGTTT	GCTGCTGCGG	GGCGTCAAGC	GCGAGGACGT	CGAGCGTGGC	60
CAGGTTGTCA	CCAAGCCCGG	CACCACCACG	CCGCACACCG	AGTTCGAAGG	CCAGGTCTAC	66
ATCCTGTCCA	AGGACGAGGG	CGGCCGGCAC	ACGCCGTTCT	TCAACAACTA	CCGTCCGCAG	72
TTCTACTTC	GCACCACCG	CGTGACCGGT	GTGGTGACAC	TGCCGGAGGG	CACCGAGATG	78
GTGATGCCC	GTGACAACAC	CAACATCTCC	GTGAAGTTG	TCCAGCCCGT	CGCCATGGAC	84
GAAGGTCTG	GTTTCGCGAT	CCGCGAGGGT	GGCCGCACCC	TGGGCGCCG	C C	89

- (2) INFORMATION FOR SEQ ID NO: 161:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 891 base pairs (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:

Section 1

- 146 -

(A)	ORGANISM:	Mycoplasma	genitalium

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 161:

AATATGATCA	CAGGTGCTGC	ACAAATGGAT	GGAGCTATTC	TAGTTGTTTC	AGCAACTGAT	60
AGTGTGATGC	CCCAAACCCG	CGAGCACATC	TTACTTGCCC	GCCAAGTAGG	GGTTCCTAAA	120
ATGGTAGTTT	TTCTAAACAA	GTGTGATATT	GCTAGTGATG	AAGAGGTACA	AGAACTTGTT	180
GCTGAAGAAG	TACGTGATCT	GTTAACTTCC	TATGGTTTTG	ATGGTAAGAA	CACTCCTATT	240
ATTTATGGCT	CAGCTTTAAA	AGCATTGGAA	GGTGATCCAA	AGTGGGAGGC	TAAGATCCAT	300
GATTTGATTA	AAGCAGTTGA	TGAATGGATT	CCAACTCCTA	CACGTGAAGT	AGATAAACCT	360
TTCTTATTAG	CAATTGAAGA	TACGATGACC	ATTACTGGTA	GAGGTACAGT	TGTTACAGGA	420
AGAGTTGAAA	GAGGTGAACT	CAAAGTAGGT	CAAGAAGTTG	AAATTGTTGG	TTTAAAACCA	480
ATTAGAAAAG	CAGTTGTTAC	TGGAATTGAA	ATGTTCAAAA	AGGAACTTGA	TTCAGCAATG	540
GCTGGTGACA	ATGCTGGGGT	ATTATTACGT	GGTGTTGAAC	GTAAAGAAGT	TGAAAGAGGT	600
CAAGTTTTAG (	CAAAACCAGG	CTCTATTAAA	CCGCACAAGA	AATTTAAAGC	TGAGATCTAT	660
GCTTTAAAGA A	AAGAAGAAGG	TGGTAGACAC	ACTGGTTTTT	TAAACGGTTA	CCGTCCTCAA	720
TTCTATTTCC (	STACCACTGA	TGTAACTGGT	TCTATTGCTT	TAGCTGAAAA	TACTGAAATG	.780
GTTCTACCTG (	TGATAATGC	TTCTATTACT	GTTGAGTTAA	TTGCTCCTAT	CGCTTGTGAA	840
AAAGGTAGTA	GTTCTCAAT	TCGTGAAGGT	GGTAGAACTG	TAGGGGCAGG	C - 1,	±14 891
(2) INFORMAT	TION FOR SE	Q ID NO: 16	2:			

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 891 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear

  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Neisseria gonorrheae
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 162:

AACATGATTA CCGGCGCCG ACAAATGGAC GGTGCAATCC TGGTATGTTC TGCTGCCGAC

GGCCCTATGC CGCAAACCCG CGAACACATC CTGCTGGCCC GTCAAGTAGG CGTACCTTAC

120

ATCATCGTGT TCATGAACAA ATGCGACATG GTCGACGATG CCGAGCTGTT CCAACTGGTT

GAAATGGAAA TCCGCGACCT GCTGTCCAGC TACGACTTCC CCGCGACGA CTGCCCGATC

240

GTACAAGGTT CCGCACTGAA AGCCTTGGAA GGCGATGCCG CTTACGAAGA AAAAATCTTC 30	00
	60
	20
	80
	40
	00
	60
	20
	80
	340
	391
(2) INFORMATION FOR SEQ ID NO: 163:	
(A) LENGTH: 891 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear  (ii) MOLECULE TYPE: DNA (genomic)  (vi) ORIGINAL SOURCE: (A) ORGANISM: Rickettsia prowazekii	ė •
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 163:	
AATATGATAA CTGGTGCCGC TCAGATGGAT GGTGCTATAT TAGTAGTTTC TGCTGCTGAT	60
	120
	180
GAGATGGAAG TAAGAGAATT ATTATCAAAA TATGGTTTCC CTGGTAATGA AATACCTATT	240
ATTAAAGGTT CTGCACTTCA AGCTTTAGAA GGAAAACCTG AAGGTGAAAA AGCTATTAAT	300
GAGTTAATGA ATGCAGTAGA TACGTATATA CCTCAGCCTA TAGAGCTACA AGATAAACCT	360
TTTTTAATGC CAATAGAGGA TGTATTTTCT ATTTCAGGCA GAGGTACCGT TGTAACTGGT	420
AGAGTGGAGT CAGGCATAAT TAAGGTGGGT GAAGAAATTG AAATAGTAGG TCTAAAAAAT	48

TCTGGAGATA ATGTCGGTAT ATTACTACGT GGTACAAAAA GAGAAGAAGT AGAAAGAGGA

- 148 -

CAAGTACTTG CAAAACCT	GG GAGCATAAAA	CCGCATGATA	AATTTGAAGC	TGAAGTGTAT	660
GTGCTTAGTA AAGAGGAA	GG TGGACGTCAT	ACCCCATTTA	CTAATGATTA	TCGCCCACAG	720
TTCTATTTTA GAACAACA	GA TGTTACCGGC	ACAATAAAAT	TGCCTTCTGA	TAAGCAGATG	780
GTTATGCCTG GAGATAAT	GC TACTTTTCA	GTAGAATTAA	TTAAGCCGAT	TGCTATGCAA	840
GAAGGGTTAA AATTCTCT	AT ACGTGAAGGT	GGTAGAACAG	TAGGAGCCGG	T	891
(2) INFORMATION FOR	CTC				

- (2) INFORMATION FOR SEQ ID NO: 164:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 891 base pairs
      - (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Salmonella typhimurium
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 164:

AACATGATCA CCGGTGCTGC TCAGATGGAC GGCGCGATCC TGGTTGTTGC TGCGACTGAC 60 GGCCCGATGC CGCAGACCCG TGAGCACATC CTGCTGGGTC GTCAGGTAGG CGTTCCGTAC 120 ATCATCGTGT TCCTGAACAA ATGCGACATG GTTGATGACG AAGAGCTGCT GGAACTGGTT 180 GAGATGGAAG TTCGCGAACT GCTGTCTCAG TACGACTTCC CGGGCGACGA CACTCCGATC 240 GTTCGTGGTT CTGCTCTGAA AGCGCTGGAA GGCGACGCAG AGTGGGAAGC GAAAATCATC 300 GAACTGGCTG GCTTCCTGGA TTCTTATATT CCGGAACCAG AGCGTGCGAT TGACAAGCCG 360 TTCCTGCTGC CGATCGAAGA CGTATTCTCC ATCTCCGGTC GTGGTACCGT TGTTACCGGT 420 CGTGTAGAGC GCGGTATCAT CAAAGTGGGC GAAGAAGTTG AAATCGTTGG TATCAAAGAG 480 ACTCAGAAGT CTACCTGTAC TGGCGTTGAA ATGTTCCGCA AACTGCTGGA CGAAGGCCGT 540 GCCGGTGAGA ACGTAGGTGT TCTGCTGCGT GGTATCAAAC GTGAAGAAAT CGAACGTGGT 600 CAGGTACTGG CTAAGCCGGG CACCATCAAG CCGCACACCA AGTTCGAATC TGAAGTGTAC 660 ATTCTGTCCA AAGATGAAGG CGGCCGTCAT ACTCCGTTCT TCAAAGGCTA CCGTCCGCAG 720 TTCTACTTCC GTACTACTGA CGTGACTGGT ACCATCGAAC TGCCGGAAGG CGTAGAGATG 780 GTAATGCCGG GCGACAACAT CAAAATGGTT GTTACCCTGA TCCACCCGAT CGCGATGGAC 840 GACGGTCTGC GTTTCGCAAT CCGTGAAGGC GGCCGTACCG TTGGCGCGGG C 891 (2) INFORMATION FOR SEQ ID NO: 165:

(i)	SEQUI	ENCE CHARACTERISTICS:
		LENGTH: 881 base pair
	(B)	TYPE: nucleic acid
	(C)	STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE: (A) ORGANISM: Shewanella putida

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 165:

60
120
180
240
300
360
420
480
540
600
660
720
78
84
88

- (2) INFORMATION FOR SEQ ID NO: 166:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 897 base pairs (B) TYPE: nucleic acid
      - (C) STRANDEDNESS: double
      - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Stigmatella aurantiaca

- 150 -

(xi)	SEQUENCE	DESCRIPTION:	SEO	ID	NO -	166
------	----------	--------------	-----	----	------	-----

AACATGATCA	CGGGCGCGGC	GCAGATGGAC	GGAGCGATTC	TGGTGGTGT	CGCGGCCGAC	60
GGCCCGATGC	CCCAGACGCG	TGAGCACATC	CTGCTGGCCA	GGCAGGTGGG	CGTGCCCTAC	120
ATCGTCGTCT	TCCTGAACAA	GGTGGACATG	CTGGACGATC	CGGAGCTGCG	CGAGCTGGTG	180
GAGATGGAGG	TGCGCGACCT	GCTCAAGAAG	TACGAGTTCC	CGGGCGACAG	CATCCCCATC	240
ATCCCTGGCA	GCGCGCTCAA	GGCGCTGGAG	GGAGACACCA	GCGACATCGG	CGAGGGAGCG	300
ATCCTGAAGC	TGATGGCGGC	GGTGGACGAG	TACATCCCGA	CGCCGCAGCG	TGCGACGGAC	360
AAGCCGTTCC	TGATGCCGGT	GGAAGACGTG	TTCTCCATCG	CAGGCCGAGG	AACGGTGGCG	420
ACGGGCCGAG	TGGAGCGCGG	CAAGATCAAG	GTGGGCGAGG	AAGTGGAGAT	CGTGGGGATC	480
CGTCCGACGC	AGAAGACGGT	CATCACGGGG	GTGGAGATGT	TCCGCAAGCT	GCTGGACGAG	540
GGCATGGCGG	GAGACAACAT	CGGAGCGCTG	CTGCGAGGCC	TGAAGCGCGA	GGACCTGGAG	600
CGTGGGCAGG	TGCTGGCGAA	CTGGGGGAGC	ATCAACCCGC	ACACGAAGTT	CAAGGCGCAG	660
GTGTACGTGC	TGTCGAAGGA	AGAGGGAGGG	CGGCACACGC	CGTTCTTCAA	GGGATACCGG	720
CCGCAGTTCT	ACTTCCGGAC	GACGGACGTG	ACCGGAACGG	TGAAGCTGCC	GGACAACGTG	780
GAGATGGTGA	TGCCGGGAGA	CAACATCGCC	ATCGAGGTGG	AGCTCATTAC	TCCGGTCGCC	840
ATGGAGAAGG	AGCTGCCGTT	CGCCATCCGT	GAGGGTGGCC	GCACGGTGGG	CGCCGGC	897
(2) INFORMA	TION FOR SE	Q ID NO: 16	7:			

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 894 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Streptococcus pyogenes (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 167:
- AACATGATCA CTGGTGCCGC TCAAATGGAC GGAGCTATCC TTGTAGTTGC TTCAACTGAT 60 GGACCAATGC CACAAACTCG TGAGCACATC CTTCTTTCAC GTCAGGTTGG TGTTAAACAC 120 CTTATCGTGT TCATGAACAA AGTTGACCTT GTTGATGACG AAGAGTTGCT TGAATTAGTT 180 GAGATGGAAA TTCGTGACCT TCTTTCAGAA TACGATTTCC CAGGTGATGA CCTTCCAGTT 240 ATCCAAGGTT CAGCTCTTAA AGCTCTTGAA GGCGACACTA AATTTGAAGA CATCATCATG

- 151 -

gaattgatgg	ATACTGTTGA	TTCATACATT	CCAGAACCAG	AACGCGACAC	TGACAAACCA	360
TTGCTTCTTC	CAGTCGAAGA	CGTATTCTCA	ATTACAGGTC	GTGGTACAGT	TGCTTCAGGA	420
CGTATCGACC	GTGGTACTGT	TCGTGTCAAC	GACGAAATCG	AAATCGTTGG	TATCAAAGAA	480
GAAACTAAAA	AAGCTGTTGT	TACTGGTGTT	GAAATGTTCC	GTAAACAACT	TGACGAAGGT	540
CTTGCAGGAG	ACAACGTAGG	TATCCTTCTT	CGTGGTGTTC	AACGTGACGA	AATCGAACGT	600
GGTCAAGTTA	TTGCTAAACC	AAGTTCAATC	AACCCACACA	CTAAATTCAA	AGGTGAAGTA	660
TATATCCTTT	CTAAAGACGA	AGGTGGACGT	CACACTCCAT	TCTTCAACAA	CTACCGTCCA	720
CAATTCTACT	TCCGTACAAC	TGACGTAACA	GGTTCAATCG	AACTTCCAGC	AGGTACAGAA	780
ATGGTTATGC	CTGGTGATAA	CGTGACAATC	AACGTTGAGT	TGATCCACCC	AATCGCCGTA	840
GAACAAGGTA	CTACTTTCTC	AATCCGTGAA	GGTGGACGTA	CTGTTGGTTC	AGGT	894

- (2) INFORMATION FOR SEQ ID NO: 168:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 897 base pairs
      - (B) TYPE: nucleic acid
      - (C) STRANDEDNESS: double (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (vi) ORIGINAL SOURCE:
    - (A) ORGANISM: Thiobacillus cuprinus
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 168:

AACATGATCA CCGGTGCGGC CCAGATGGAC GGCGCCATCC TGGTCGTGTC CGCCGCCGAC 60 GGCCCCATGC CCCAAACCCG CGAGCACATC CTGCTGGCGC GTCAGGTGGG CGTGCCCTAC 120 ATCATCGTGT TCCTCAACAA GTGCGACATG GTCGACGACG CCGAGCTGCT CGAACTCGTC 180 GAGATGGAAG TGCGCGAGCT GCTGTCCAAG TACGACTTCC CCGGTGACGA CACCCCCATC 240 ATCAAGGGCT CGGCCAAGCT GGCCCTCGAA GGCGACAAGG GCGAACTGGG CGAAGGCGCC 300 ATTCTCAAGC TGGCCGAGGC CCTGGACACC TACATCCCCA CGCCCGAGCG GGCCGTCGAC 360 GGCGCGTTCC TCATGCCCGT GGAAGACGTG TTCTCCATCT CCGGGCGCGG CACGGTGGTC 420 ACCGGGCGTG TGGAGCGCGG CATCATCAAG GTCGGCGAGG AAATCGAGAT TGTCGGCCTC 480 AAGCCCACCC TCAAGACCAC CTGCACCGGC GTGGAAATGT TCAGGAAGCT GCTCGACCAG 540 GGCCAGGCCG GCGACAACGT CGGCATCTTG CTGCGCGGCA CCAAGCGCGA GGAAGTCGAG 600 CGCGGCCAGG TGCTGTGCAA ACCCGGCTCG ATCAAGCCCC ACACCCACTT CACCGCCGAG 660 - 152 -

GTGTACGTGC TGAGCAAGGA CGAGGGCGGC CGCCACACCC CCTTCTTCAA CAACTACCGC	720
CCGCAGTTCT ACTTCCGCAC CACCGACGTC ACCGGCGCCA TCGAACTGCC CAAGGACAAG	780
GARATGGTCA TGCCCGGCGA TARTGTGAGC ATCACCGTCA AGCTCATCGC CCCCATCGCC	840
ATGGAAGAAG GCCTGCGCTT CGCCATCCGC GAAGGCGGCC GCACCGTCGG CGCCGGC	8,97
(2) INFORMATION FOR SEQ ID NO: 169:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 894 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(vi) ORIGINAL SOURCE: (A) ORGANISM: Treponema pallidum	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 169:	
AATATGATCA CGGGTGCTGC GCAGATGGAC GGTGGTATTC TCGTCGTGTC TGCGCCTGAC	60
GGCGTTATGC CACAGACGAA GGAGCATCTT CTGCTCGCCC GTCAGGTTGG TGTTCCCTCC	120
ATCATTGTTT TTTTGAACAA GGTTGATTTG GTTGATGATC CTGAGTTGCT AGAGCTGGTG	180
GAAGAAGAGG TGCGTGATGC GCTTGCTGGA TATGGGTTTT CGCGTGAGAC GCCTATCGTC	240
AAGGGGTCTG CGTTTAAAGC TCTGCAGGAT GGCGCTTCCC CGGAGGATGC AGCTTGTATT	300
GAGGAACTGC TTGCGGCCAT GGATTCCTAC TTTGAAGACC CAGTGCGTGA CGACGCAAGA	360
CCTTTCTTGC TCTCTATCGA GGATGTGTAC ACTATTTCTG GGCGTGGTAC CGTTGTCACG	420
GGGCGCATCG AATGTGGGGT AATTAGTCTG AATGAAGAGG TCGAGATCGT CGGGATTAAG	480
CCCACTAAGA AAACAGTGGT TACTGGCATT GAGATGTTTA ATAAGTTGCT TGATCAGGGA	540
ATTGCAGGTG ATAACGTGGG GCTGCTTTTG CGCGGGGTGG ATAAAAAAGA GGTTGAGCGC	600
GGTCAGGTGC TTTCTAAGCC CGGTTCTATT AAGCCACACA CCAAGTTTGA GGCGCAGATC	660
TACGTGCTCT CTAAGGAAGA GGGTGGCCGT CACAGTCCTT TTTTTCAAGG TTATCGTCCG	720
CAGTTTTATT TTAGAACTAC TGACATTACC GGTACGATTT CTCTTCCTGA AGGGGTAGAC	780
ATGGTGAAGC CGGGGGATAA CACCAAGATT ATAGGTGAGC TCATCCACCC GATAGCTATG	840
GACAAGGGTC TGAAGCTTGC GATTCGTGAA GGGGGGCGCA CTATTGCTTC TGGT	894
(2) INFORMATION FOR SEQ ID NO: 170:	

(i) SEQUENCE CHARACTERISTICS:

	LENGTH:			
(B)	TYPE: n	ıcleic	aci	d
		MIDGO.	2-1	hl a

- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Ureaplasma urealyticum
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 170:

AATATGATTA	CAGGGGCAGC	ACAAATGGAT	GGAGCAATTT	TAGTTATTGC	TGCATCTGAT	60
GGGGTTATGG	CTCAAACTAA	AGAACATATT	TTATTAGCAC	GTCAAGTTGG	TGTTCCAAAA	120
ATCGTTGTTT	TCTTAAACAA	ATGTGATTTC	ATGACAGATC	CAGATATGCA	AGATCTTGTT	180
GAAATGGAAG	TTCGTGAATT	ATTATCTAAA	TATGGATTTG	ATGGCGATAA	CACACCAGTT	240
ATTCGTGGTT	CAGGTCTTAA	GGCTTTAGAA	GGAGATCCAG	TTTGAGAAGC	AAAAATTGAT	300
GAATTAATGG	ACGCAGTTGA	TTCATGAATT	CCATTACCAG	AACGTAGTAC	TGACAAACCA	360
TTCTTATTAG	CAATTGAAGA	TGTATTCACA	ATTTCAGGAC	GTGGTACAGT	AGTAACTGGA	420
CGTGTTGAAC	GTGGTGTATT	AAAAGTTAAT	GATGAGGTTG	AAATTGTTGG	TCTAAAAGAC	480
ACTCAAAAAA	CTGTTGTTAC	AGGAATTGAA	ATGTTTAGAA	AATCATTAGA	TCAAGCTGAA	540
GCTGGTGATA	ATGCTGGTAT	TTTATTACGT	GGTATTAAAA	AAGAAGATGT	TGAACGTGGT	600
CAAGTACTTO	TAAAACCAGG	ATCAATTAAA	CCTCACCGTA	CTTTTACTGC	TAAAGTTTAT	660
ATTCTTAAAA	AAGAAGAAGG	TGGACGTCAT	ACACCTATTG	TTTCAGGATA	CCGTCCACAA	720
TTCTATTTT	A GAACAACAGA	TGTAACAGGI	GCTATTTCAT	TACCTGCTGG	TGTTGATTTG	780
GTTATGCCAC	GTGATGACG	TGAAATGACI	GTAGAATTAA	TTGCTCCAGT	TGCGATTGAA	840
GATGGATCT	A AATTCTCAAT	CCGTGAAGG	GGTAAAACTG	TAGGTCATGG	T	891

- (2) INFORMATION FOR SEQ ID NO: 171:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 909 base pairs (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: double
      - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)

  - (vi) ORIGINAL SOURCE: (A) ORGANISM: Wolinella succinogenes
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 171:

a lower of

The first winds are the state of the state o

AACATGATT	A CAGGTGCTGC	TCAAATGGAT	GGCGCGATTC	TTGTTGTTTC	TGCGGCGGAT	60
GGCCCCATG	CCCAAACTAG	GGAGCACATT	CTTCTTTCTC	GACAAGTAGG	CGTTCCTTAC	120
ATCGTGGTTT	TCTTGAACAA	AGAAGATATG	GTTGATGACG	CTGAGCTTCT	TGAGCTTGTT	180
GAAATGGAAG	TTAGAGAACT	TCTTAGCAAC	TACGACTTCC	CTGGAGATGA	CACTCCTATC	240
GTTGCAGGTT	CCGCTCTTAA	AGCTCTTGAA	GAGGCTAACG	ACCAGGAAAA	TGTTGGCGAG	300
TGGGGCGAGA	AAGTATTGAA	GCTTATGGCT	GAGGTTGACC	GATATATTCC	TACGCCTGAG	360
CGAGATGTGG	ATAAGCCTTT	CCTTATGCCT	GTTGAAGACG	TATTCTCCAT	CGCGGGTCGT	. 420
GGAACCGTTG	TGACAGGAAG	AATTGAAAGA	GGCGTGGTTA	AAGTCGGTGA	CGAAGTAGAA	480
ATCGTTGGTA	TCCGAAACAC	ACAAAAAACA	ACCGTAACTG	GCGTTGAGAT	GTTCCGAAAA	540
GAGCTCGACA	AGGGTGAGGC	GGGTGACAAC	GTTGGTGTTC	TTTTGAGAGG	CACCAAGAAA	600
GAAGATGTTG	AGAGAGGTAT	GGTTCTTTGT	AAAATAGGTT	CTATCACTCC	TCACACTAAC	660
TTTGAAGGTG	AAGTTTACGT	TCTTTCCAAA	GAGGAAGGCG	GACGACACAC	TCCATTCTTC	720
AATGGATACC	GACCTCAGTT	CTATGTTAGA	ACTACAGACG	TTACCGGTTC	TATCTCTCTT	780
CCTGAGGGCG	TAGAGATGGT	TATGCCTGGT	GACAACGTTA	AGATCAATGT	TGAGCTTATC	840
GCTCCTGTAG	CCCTCGAAGA	GGGAACACGA	TTCGCGATCC	GTGAAGGTGG	TCGAACCGTT	900
GGTGCGGGT						909

- (2) INFORMATION FOR SEQ ID NO: 172:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 26 base pairs
    - (B) TYPE: nucleic acid
    - (C) STRANDEDNESS: single
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: DNA (genomic)
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION: 6
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:12
    - (D) OTHER INFORMATION:/note= "n = inosine"
  - (ix) FEATURE:
    - (A) NAME/KEY: misc\_feature
    - (B) LOCATION:18
    - (D) OTHER INFORMATION:/note= "n = inosine"

-	1	5	5	-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 172:	
TARTCNGTRA ANGCYTCNAC RCACAT	26
(2) INFORMATION FOR SEQ ID NO: 173:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 21 base pairs (B) TYPE: nucleic acid (C) STRANDENNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 173:	
TCTTTAGCAG AACAGGATGA A	21
(2) INFORMATION FOR SEQ ID NO: 174:	
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 base pairs (B) TYPE: nucleic acid (C) STRANDENNESS: single (D) TOPOLOGY: linear	
(ii) MOLECULE TYPE: DNA (genomic)	
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 174:	
GAATAATTCC ATATCCTCCG	20



## **CLAIMS**

10

20

## What is claimed is:

- A method using probes and/or amplification primers which are specific, ubiquitous and sensitive for determining the presence and/or amount of nucleic acids:
- 5 from a bacterial antibiotic resistance gene selected from the group consisting of bla<sub>tem</sub>, bla<sub>tem</sub>, bla<sub>cen</sub>, bla<sub>cen</sub>, bla<sub>cen</sub>, bla<sub>cen</sub>, bla<sub>cen</sub>, bla<sub>cen</sub>, bla<sub>cen</sub>, bla<sub>cen</sub>, add(6'), aacC1, aacC2, aacC3, aac6'-lla, aacA4, aad(6'), vanA, vanB, vanC, msrA, satA, aac(6')-aph(2''), vat, vga, ermA, ermB, ermC, mecA, int and sul, and
  - from specific bacterial and fungal species selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species, and Candida species,

in any sample suspected of containing said bacterial and/or fungal nucleic acids.

15 wherein each of said nucleic acid or variant or part thereof comprises a selected target region hybridizable with said probes or primers;

said method comprising the following steps: contacting said sample with said probes or primers and detecting the presence and/or amount of hybridized probes or amplified products as an indication of the presence and/or amount of said specific bacterial and/or fungal species and bacterial antibiotic resistance genes.

- A method according to claim 1, which further makes use of probes and/or primers which are specific, ubiquitous and sensitive for determining the presence and/or amount of nucleic acids from any bacterium or fungus.
- The method of claim 1, which is performed directly from a test sample.
- The method of claim 1, which is performed directly from a test sample consisting of a bacterial and/or fungal culture or suspension.
  - The method of claim 1, wherein said nucleic acids are all detected under uniform hybridization or amplification conditions.
- 6. The method of claim 1, wherein said nucleic acids are amplified by a method selected from the group consisting of:
  - a) polymerase chain reaction (PCR),
  - b) ligase chain reaction (LCR),
  - c) nucleic acid sequence-based amplification (NASBA),

10

15

20

25

30

- d) self-sustained sequence replication (3SR),
- e) strand displacement amplification (SDA),
- f) branched DNA signal amplification (bDNA).
- g) transcription-mediated amplification (TMA),
- h) cycling probe technology (CPT),
- i) nested PCR, and
- i) multiplex PCR.
- 7. The method of claim 6, wherein said nucleic acids are amplified by PCR.
- 8. The method of claim 7, wherein the PCR protocol achieves within one hour under uniform amplification conditions the determination of the presence of said nucleic acids by performing for each amplification cycle an annealing step of thirty seconds at 45-55°C and a denaturation step of only one second at 95°C without any time specifically allowed to an elongation step.
- 9. A method for the detection, identification and/or quantification of a microorganism selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species and Candida species, directly from a test sample or from bacterial and/or fungal cultures, which comprises the following steps:
  - a) depositing and fixing on an inert support or leaving in solution the said microorganism DNA of the sample or of a substantially homogeneous population of said microorganism isolated from this sample, or
  - inoculating said sample or said substantially homogeneous population of microorganism isolated from this sample on an inert support, and lysing in situ said inoculated sample or said isolated microorganism to release the said microorganism DNA.

said microorganism DNA being made in a substantially single-stranded form;

b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleic acid which nucleotide sequence is selected from the group consisting of SEQ ID NOs: 26, 27, 28, 29, 30, 120, 131 to 134, 31, 140 to 143, 32 to 36, 120 to 124, a sequence complementary thereof, a part thereof having at least 12 nucleotides in length, and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of *Enterococcus faecium*, *Listeria* 

15

20

monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species and Candida species, respectively, under conditions such that the nucleic acid of said probe can selectively hybridize with said microorganism DNA, whereby a hybridization complex is formed; and

- c) detecting the presence of said hybridization complex on said inert support or in said solution as an indication of the presence and/or amount of said microorganism, in said test sample.
- 10. A method for detecting the presence and/or amount of a microorganism selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species and Candida species, in a test sample which comprises the following steps:
  - a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said microorganism DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NOs: 26, 27, 28, 29, 30, 120, 131 to 134, 31, 140 to 143, 32 to 36, 120 to 124, respectively with regard to said microorganism, a sequence complementary thereof, and a variant thereof:
- 25 b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of said microorganisms, in said test
   sample.
  - 11. The method of claim 10, wherein said pair of primers is defined in SEQ ID NOs: 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, 11 and 12, 13 and 14, 15 and 16, 17 to 20, 21 and 22, respectively, for each of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species and Streptococcus species.

10

15

20

25

30

- 12. A method for detecting the presence and/or amount of any bacterium directly from a test sample or a bacterial culture, which comprises the following steps:
- a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogeneous population of bacteria isolated from this sample, or

inoculating said sample or said substantially homogeneous population of bacteria isolated from this sample on an inert support, and lysing *in situ* said inoculated sample or isolated bacteria to release the bacterial DNA,

said bacterial DNA being made in a substantially single-stranded form;

- b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleic acid which nucleotide sequence is selected from the group consisting of SEQ ID NOs: 118, 119, 125 to 171, a sequence complementary thereof, a part thereof having at least 12 nucleotides in length, and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of any bacterial species, under conditions such that the nucleic acid of said probe can selectively hybridize with said bacterial DNA, whereby a hybridization complex is formed; and
  - c) detecting the presence of said hybridization complex on said inert support or in said solution as an indication of the presence and/or amount of any bacterium in said test sample.
  - 13. A method for detecting the presence and/or amount of any bacterium in a test sample which comprises the following steps:
  - a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of any bacterial DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NO: 118, 119, 125 to 171, a sequence complementary thereof, and a variant thereof;
  - b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as
   an indication of the presence and/or amount of any bacterium in said test sample.

10

15

20

25

30

- The method of claim 13, wherein said pair of primers is defined in SEQ ID NOs:
   and 24.
- 15. A method for obtaining *tuf* sequences from any bacterium directly from a test sample or a bacterial culture, which comprises the following steps:
- a) treating said sample with an aqueous solution containing a pair of primers having a sequence selected within the nucleotide sequences defined in SEQ ID NOs: 107 and 108, a part thereof having at least 12 nucleotides in length, a sequence complementary thereof, and a variant thereof, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial tuf gene that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template;
- b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
  - c) detecting the presence and/or amount of said amplified target sequence; and
- d) determining the nucleotide sequence of the said amplified target sequence by using any DNA sequencing method.
- 16. A method for detecting the presence and/or amount of any fungus directly from a test sample or a fungal culture, which comprises the following steps:
- a) depositing and fixing on an inert support or leaving in solution the fungal DNA of the sample or of a substantially homogeneous population of fungi isolated from this sample, or

inoculating said sample or said substantially homogeneous population of fungi isolated from this sample on an inert support, and lysing *in situ* said inoculated sample or isolated fungi to release the fungal DNA,

said fungal DNA being made in a substantially single-stranded form;

b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleotide sequence selected from the group consisting of SEQ ID NOs: 120 to 124, a sequence complementary thereof, a part thereof having at least 12 nucleotides in length, and a variant thereof, which specifically and ubiquitously anneals with strains or representatives of any fungus, under conditions such that the nucleic acid of said probe can selectively hybridize with said fungal DNA, whereby a hybridization complex is formed; and

c) detecting the presence of said hybridization complex on said inert support or SUBSTITUTE SHEET (RULE 26)

10

15

20

25

30

35



in said solution as an indication of the presence and/or amount of any fungus in said test sample.

- 17. A method for detecting the presence and/or amount of any fungus in a test sample which comprises the following steps:
- a) treating said sample with an aqueous solution containing at least one pair of oligonucleotide primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of any fungal DNA that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NOs: 120 to 124, a sequence complementary thereof, and a variant thereof;
- b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of the presence and/or amount of any fungus in said test sample.
- 18. A method for obtaining *tuf* sequences from any fungus directly from a test sample or a fungal culture, which comprises the following steps:
- a) treating said sample with an aqueous solution containing a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 109 and 172, a part thereof having at least 12 nucleotides in length, a sequence complementary thereof, and a variant thereof, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said fungal tuf gene that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template;
  - b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
    - c) detecting the presence and/or amount of said amplified targ t sequence; and
    - d) determining the nucleotide sequence of the said amplified target sequence by using any DNA sequencing method.
- 19. A method as defined in claim 1, which comprises the evaluation of the presence of a bacterial resistance mediated by a bacterial antibiotic resistance gene selected

10

15

20

25

30

from the group consisting of bla<sub>box</sub>, blaZ, aac6'-IIa, ermA, ermB, ermC, vanB, vanC, directly from a test sample or a bacterial culture, which comprises the following steps:

 a) depositing and fixing on an inert support or leaving in solution the bacterial DNA of the sample or of a substantially homogeneous population of bacteria isolated from this sample, or

inoculating said sample or said substantially homogeneous population of bacteria isolated from this sample on an inert support, and lysing in situ said inoculated sample or isolated bacteria to release the bacterial DNA.

said bacterial DNA being made in a substantially single-stranded form;

- b) contacting said single-stranded DNA with a probe, said probe comprising at least one single-stranded nucleotide sequence having at least 12 nucleotide in length is selected from the group consisting of SEQ ID NOs: 110, 111, 112, 113, 114 115, 116, 117, a sequence complementary thereof, and a variant thereof, which specifically hybridizes with said bacterial antibiotic resistance gene, respectively; and
- c) detecting the presence of a hybridization complex as an indication of a bacterial resistance mediated by said one of said bacterial antibiotic resistance genes.
- 20. A method as defined in claim 1, which comprises the evaluation of the presence of a bacterial resistance mediated by a bacterial antibiotic resistance gene selected from the group consisting of bla<sub>oxev</sub> blaZ, aac6'-lla, ermA, ermB, ermC, vanB, vanC, directly from a test sample or a bacterial culture, which comprises the following steps:
- a) treating said sample with an aqueous solution containing at least one pair of primers having at least 12 nucleotides in length, one of said primers being capable of hybridizing selectively with one of the two complementary strands of said bacterial antibiotic resistance gene that contains a target sequence, and the other of said primers being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template, said at least one pair of primers being chosen from a nucleotide sequence within the group consisting of SEQ ID NOs: 110, 111, 112, 113, 114, 115, 116, 117, respectively with regard to said bacterial antibiotic resistance gene, a sequence complementary thereof, and a variant thereof;
- b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance mediated by one of said bacterial antibiotic resistance genes.

  SUBSTITUTE SHEET (RULE 26)

10

15

20

25



- 21. A method as defined in claim 1, which comprises the evaluation of the presence of a bacterial resistance gene selected from the group consisting of  $bla_{low}$ ,  $bla_{low$
- a) treating said sample with an aqueous solution containing at least one pair of primers having a sequence selected in the group consisting of SEQ ID NOs: 37 to 40, 41 to 44, 45 to 48, 49 and 50, 51 and 52, 53 and 54, 55 and 56, 57 and 58, 59 to 60, 61 to 64, 65 and 66, 173 and 174, 67 to 70, 71 to 74, 75 and 76, 77 to 80, 81 and 82, 83 to 86, 87 and 88, 89 and 90, 91 and 92, 93 and 94, 95 and 96, 97 and 98, 99 to 102, 103 to 106, a part thereof having at least 12 nucleotides in length, a sequence complementary thereof, a variant thereof, and mixtures thereof, one of said primers of said pair being capable of hybridizing selectively with one of the two complementary strands of its respective bacterial antibiotic resistance gene that contains a target sequence, and the other of said primers of said pairs being capable of hybridizing with the other of said strands so as to form an extension product which contains the target sequence as a template;
- b) synthesizing an extension product of each of said primers, said extension product containing the target sequence, and amplifying said target sequence, if any, to a detectable level; and
- c) detecting the presence and/or amount of said amplified target sequence as an indication of a bacterial resistance mediated by one of said bacterial antibiotic resistance genes.
- 22. A nucleic acid having the nucleotide sequence of any one of SEQ ID NOs: 26 to 36, 110 to 171, a part thereof, a sequence complementary thereof, and variant thereof which, when in single-stranded form, ubiquitously and specifically hybridizes with a target bacterial or fungal DNA as a probe or as a primer.
- 23. An oligonucleotide having the nucleotide sequence of any one of SEQ ID NOs: 1 to 25, 37 to 109, 172 to 174, a part thereof, a sequence complementary thereof, and variant thereof, which ubiquitously and specifically hybridizes with a target bacterial or fungal DNA as a probe or as a primer.
- 24. A recombinant plasmid comprising a nucleic acid as defined in claim 22.
- 25. A recombinant host which has been transformed by a recombinant plasmid according to claim 24.
- 35 26. A recombinant host according to claim 25 wherein said host is Escherichia coli.
  - 27. A diagnostic kit for the detection and/or quantification of the nucleic acids of any SUBSTITUTE SHEET (RULE 26)

20

25

30

35

combination of the microbial species and/or genera selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species and Candida species, comprising any suitable combination of probes of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 26 to 36, 120 to 124, 131 to 134, 140 to 143, sequences complementary thereof, and variants thereof.

- 28. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the microbial species and/or genera selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species, Streptococcus species, Streptococcus species, Streptococcus species, Streptococcus species, Comprising any suitable combination of primers of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 26 to 36, 120 to 124, 131 to 134, 140 to 143, sequences complementary thereof, and variants thereof.
  - 29. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the microbial species and/or genera selected from the group consisting of Enterococcus faecium, Listeria monocytogenes, Neisseria meningitidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Candida albicans, Enterococcus species, Neisseria species, Staphylococcus species and Streptococcus species, comprising any suitable combination of primers selected from the group consisting of SEQ ID NOs: 1 to 22, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof.
    - 30. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes selected from the group consisting of bla<sub>cosp</sub>, blaZ, aac6'-Ila, ermA, ermB, ermC, vanB, vanC, comprising any suitable combination of probes of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 110 to 117, sequences complementary thereof, and variants thereof.
  - 31. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes selected from the group consisting of bla<sub>oxa</sub>, blaZ, aac6'-Ila, ermA, ermB, ermC, vanB, vanC, comprising any suitable combination of primers of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 110 to 117, sequences complementary thereof, and variants thereof.

10

15



32. A diagnostic kit for the detection and/or quantification of the nucleic acids of any combination of the bacterial resistance genes selected from the group consisting of bla<sub>tor</sub> b

A diagnostic kit for the detection and/or quantification of the nucleic acids of any bacterium and/or fungus, comprising any combination of probes of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof.

- 34. A diagnostic kit for the detection and/or quantification of the nucleic acids of any bacterium and/or fungus, comprising any suitable combination of primers of at least 12 nucleotides in length selected from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof.
- 35. A diagnostic kit for the detection and/or quantification of the nucleic acids of any bacterium, comprising a pair of primers having a sequence selected within th nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof.
- 20 36. A diagnostic kit, as defined in claim 27, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.
- 25 37. A diagnostic kit, as defined in claim 28, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.
- 30 38. A diagnostic kit, as defined in claim 29, further comprising a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium.
- 35 39. A diagnostic kit, as defined in claim 27, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from



the group consisting of SEQ ID NOs: 110 to 117, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterial antibiotic resistance gene selected from the group consisting of bla<sub>out</sub>, blaZ, aac6'-Ila, ermA, ermB, ermC, vanB, vanC.

- 5 40. A diagnostic kit, as defined in claim 28, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 110 to 117, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterial antibiotic resistance gene selected from the group consisting of bla<sub>ous</sub>, blaZ, aac6'-lla, ermA, ermB, ermC, vanB, vanC.
  - 41. A diagnostic kit, as defined in claim 29, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 37 to 106, 173 and 174, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterial antibiotic resistance gene selected from the group consisting of  $bla_{torn}$   $bla_{ror}$   $bla_{torn}$   $bla_{ous}$  blaZ, aadB, aacC1, aacC2, aacC3, aacA4, aac6-lla, aad(6-lla, aad(6-lla, aad(6-lla), ama(6-lla), ama(6-lla), ama(6-lla), ama(6-lla), ama(6)-ama
  - 42. A diagnostic kit, as defined in claim 30, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.
- 43. A diagnostic kit, as defined in claim 31, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.
- 44. A diagnostic kit, as defined in claim 32, further comprising a pair of primers having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium.
- 45. A diagnostic kit, as defined in claim 39, further comprising any combination of probes of at least 12 nucleotides in length selected within a nucleotide sequence from the group consisting of SEQ ID NOs: 118 to 171, sequences complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic

15

10

acids of any bacterium and/or fungus.

- 46. A diagnostic kit, as defined in claim 40, further comprising any suitable combination of primers of at least 12 nucleotides in length selected within a nucleotid sequence from the group consisting of SEQ ID NOs: 118 to 171, sequenc s complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium and/or fungus.
- 47. A diagnostic kit, as defined in claim 41, further comprising a pair of prim rs having a sequence selected within the nucleotide sequence defined in SEQ ID NOs: 23 and 24, parts thereof having at least 12 nucleotides in length, sequenc s complementary thereof, and variants thereof, for the simultaneous detection and/or quantification of nucleic acids of any bacterium.

